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INHERITANCE OF THE X CHROMOSOME IN EXCEPTIONAL MALES OF SCIARA OCELLARIS (DIPTERA)

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Received October 26, 1942

INTRODUCTION

THE present paper represents an attempt to throw further light on the subject of sex determination and the sex chromosome mechanism in Sciara. Earlier work in this field has been reviewed by METZ (1938), and a brief review here will suffice. In some species of Sciara and in some strains of other species, females produce progenies, or families, which are all or nearly all of one sex. In such a progeny the few individuals of the other sex which may occur are called exceptional males (in female progenies) or exceptional females (in male progenies). It was found by METZ that males normally get their somatic X chromosome from the female parent. CROUSE and SMITH-STOCKING (1938) state that there are many cases of irregularity in the inheritance of the X chromosome in males when the yellow mutant strain of *S. ocellaris* is crossed with wild type. The irregularity does not appear to be causually associated with the mutant gene. A sex-linked mutant gene is necessary as a marker in order to detect the irregularity, which is presumably occurring also when a marker is not present. The evidence given below indicates that the inheritance of the X chromosome is irregular only in exceptional males and, to some extent, in males of "bisexual" progenies which are predominantly female, and further that exceptional males which retain the paternal X chromosome are sterile.

In *S. coprophila* female-producing females differ from male-producing females by the possession of a modified X chromsome, designated X′ (X prime). Female producers are heterozygous (X′X), while male producers are homozygous for the normal X chromosome (XX). All females which receive the X′ chromosome are female producers. The two types of females are apparently produced in a 1:1 ratio. In *S. ocellaris*, on the other hand, the ratio of males to females within a progeny varies greatly. Some strains behave much like *S. coprophila* and give "unisexual" progenies. Others give both sexes in varying proportions. It has been found in studies as yet unpublished that some females from a "bisexual" strain may give "unisexual" progenies while their sisters yield progenies of various proportions of males and females all the way to a ratio of 1:1. Generally in such cases the "unisexual" progenies of sisters are of the same sex and the "bisexual" progenies of their sisters tend to have more individuals of this sex than of the other sex. In other cases the progenies of sisters may be predominantly about the 1:1 ratio, or the mode may apparently be around any arbitrary ratio, or the distribution may be bi-modal. In both "unisexual" and "bisexual" strains of *S. ocellaris* male producers and female producers frequently are not produced in a 1:1 ratio. Tests for the X′ chromosome in *S. ocellaris* show that it does not exist sharply distinguishable

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from the X chromosome as in *S. coprophila*. Although the studies are not yet complete, they indicate that X chromosomes have various tendencies and potencies in the X′ direction and that factors which influence sex are present also on the autosomes.

In the oögenesis of Sciara the maturation divisions show no unusual characteristics, but in spermatogenesis meiosis is quite atypical. Two X chromosomes occur in each sperm. These chromosomes are genetically equal, the products of the division of a single X chromosome which went precociously to one pole of the spindle in the second meiotic division. The chromosomes which go to the other pole are budded off. Thus the fertilized egg contains three X chromosomes, of which one or two are eliminated from the somatic tissue in early cleavages, depending on whether the individual is to become a female or male. Normally one of the two X chromosomes from the sperm is eliminated in individuals to become females, while in those to become males both X chromosomes contributed by the sperm are eliminated, leaving only the one of maternal origin.

Elimination of a chromosome also takes place in the germ line of the developing embryo, but this differs from that in the soma. One X chromosome is normally eliminated in both males and females, and this chromosome is ordinarily one of the two paternal X chromosomes. Thus the spermatogonia and oögonia each have two X chromosomes, one of maternal and the other of paternal derivation. Males transmit only the one of maternal origin.

In "unisexual" strains the cytoplasm of the eggs appears to be conditioned by the genetic constitution of the mother so as to pre-determine to some extent the nature of chromosome elimination from the soma during development. Thus the eggs of male-producing females show a tendency to eliminate the two paternal X chromosomes during development, yielding males. In the female-producing females the tendency is to eliminate only one X chromosome from the soma. An exceptional male is produced by an egg which would be expected to eliminate only one X chromosome from the soma but which actually eliminated two.

Irregularities in chromosome elimination from the soma can be detected only when the zygote is heterozygous for a sex linked factor. There are several ways in which such irregularities could occur in males. Instead of the selective elimination of paternal X chromosomes just mentioned, selection might be at random among the three X chromosomes. If the female parent is homozygous and different from the male parent, this would result in a 2:1 ratio of paternal and maternal phenotypes, respectively. This may be more easily visualized by illustrating it as follows. (Since the two X chromosomes contributed by the male are the product of the division of one chromosome during spermatogenesis, only one is represented in the male.) $y/y\,♀ \times Y♂ \rightarrow yYY$ zygotes$\rightarrow 2Y:1y$ among the phenotypes of the male offspring. If the female parent is heterozygous the ratio of paternal to maternal phenotypes would be 5:1. $Y/y\,♀ \times Y♂ \rightarrow YYY + yYY$ zygotes$\rightarrow 5Y:1y$, or $Y/y\,♀ \times y♂ \rightarrow Yyy + yyy$ zygotes$\rightarrow 5y:1Y$.

If one X chromosome contributed by the male were eliminated as usual and selection were at random between the other paternal and the maternal X

chromosome, the ratio of phenotypes among the male offspring would be 1:1 when the female is homozygous and different from the male, and 3:1 when the female is heterozygous. This may perhaps be best represented by putting in parenthesis the symbol for one of the paternal X chromosomes, leaving random selection between the other two. y/y ♀ × Y ♂ → $yY(Y)$ zygotes → 1 y : 1 Y among the phenotypes of the male offspring. Y/y ♀ × Y ♂ → $YY(Y) + yY(Y)$ zygotes → 3 Y : 1 y, or Y/y ♀ × y ♂ → $Yy(y) + yy(y)$ zygotes → 3 y : 1 Y. Other ratios would occur if there were selection favoring the retention of an X chromosome from one parent or the other.

EXPERIMENTAL DATA

Table 1 shows the ratios expected on the basis of the kinds of selection mentioned and gives the values observed. The two mutant genes concerned

TABLE 1

Ratios expected on the basis of four types of selection of the somatic X chromosome of male offspring, and the values observed. The types of selection listed are: I. Random selection among all three X chromosomes present in the fertilized egg. II. Random selection between the X chromosome of maternal origin and one of the X chromosomes of paternal origin. III. Selection of the maternal X chromosome. IV. Selection of a paternal X chromosome.

Genotypes of parents	♀ ♂ $y/y \times Y$	♀ ♂ $Y/Y \times y$	♀ ♂ $Y/y \times Y$	♀ ♂ $Y/y \times y$	♀ ♂ $yC/yc \times yc$	♀ ♂ $YC/yC \times yc$
Phenotypes of offspring	Y : y	Y : y	Y : y	Y : y	yC : yc	YC : yC : yc
Expected ratios						
I	2 : 1	1 : 2	5 : 1	1 : 5	1 : 5	1 : 1 : 4
II	1 : 1	1 : 1	3 : 1	1 : 3	1 : 3	1 : 1 : 2
III	0 : 1	1 : 0	1 : 1	1 : 1	1 : 1	1 : 1 : 0
IV	1 : 0	0 : 1	1 : 0	0 : 1	0 : 1	0 : 0 : 1
Observed values	F 23, 1 G 35, 2	A 29, 31	A 29, 34 F 11, 0	F 3, 45 G 5, 29	Ja 6, 12 Jb 35, 36	Ja 6, 3, 3 Jb 59, 55, 4

are sex-linked recessives, yellow (body) and curled (wing). Since exceptional males are few in number, the values represent males from several groups, each group designated by a letter. The female parents yielding the flies in each group except A were sisters. In group A the males represent several generations of closely related flies.

An observed ratio may be compared with an expected ratio by the method of the binomial expansion or the method of chi-square, both too familiar to warrant description. An observed ratio is said to be significantly different from an expected ratio when the probability of obtaining it or a ratio farther from the expected is not greater than one chance in twenty. The difference between them is said to be highly significant when the probability is not greater than one chance in a hundred. The method of the binomial expansion is the more accurate. The inaccuracy of the chi-square method is considerably greater when the numbers involved are small, and in such cases the binomial expansion should be employed. When the numbers are large the chi-square method is

usually sufficient to ascertain significance, and the binomial expansion need be used only as a check in borderline cases.

In group A in table 1, the exceptional males from the cross *Y/Y* by *y* fit very well the 1:1 ratio (29*Y*, 31*y*). They are significantly and nearly highly significantly different from the 1:2 ratio. It seems therefore that they indicate the elimination of one of the paternal X chromosomes from the fertilized egg and random selection between the other two. In the cross *Y/y* by *Y* for the same group the ratio is again nearly 1:1 (29*Y*, 34*y*), but here it indicates a strong selection for the X chromosome of maternal origin. Its difference from both the 5:1 and 3:1 ratios is highly significant. The reason for the lack of uniformity in the results of the two kinds of crosses in this group is not known.

In group F there seems to be a strong selection favoring an X chromosome of paternal origin in the cross *y/y* by *Y*. The numbers (23*Y*, 1*y*) are highly significant in their difference from both the 2:1 and 1:1 ratios. In the other two crosses (*Y/y* by *Y*, 11*Y*, 0*y*; and *Y/y* by *y*, 3*Y*, 45*y*) the trend is also strongly in this direction. The first is not significantly different from the 5:1 ratio expected on the basis of random assortment of all three chromosomes. The second differs significantly from the 1:5 ratio (by the method of the binomial expansion, though not by chi-square). The first differs significantly from 3:1 (method of binomial expansion) and highly so from 1:1. The second differs highly significantly from both 1:3 and 1:1. The tendency in this group as a whole appears to be toward strong selection of a paternal X chromosome.

In group G the trend is similar to that in group F. In the cross *y/y* by *Y* (35*Y*, 2*y*) there is no question of it, while in the cross *Y/y* by *y* (5*Y*, 29*y*) all the ratios are probable except 1:1, which is highly improbable. Groups F and G are very closely related, since the female parents of the two sets of sisters which produced these progenies were sisters and the male parents were brothers.

In group J the progenies which were predominantly female were essentially "bisexual," while those which were predominantly male were "unisexual." An arbitrary limit was drawn to separate the "unisexual" from the "bisexual" progenies. The males of those in which there were less than ten males per hundred females are listed in table 1 as Ja, while the males of those in which there were ten or more males per hundred females are listed as Jb. This seems to be a fairly satisfactory distribution. In "unisexual" strains there are generally not more than three of four exceptional males in a progeny, when any, but the progenies are usually less than a hundred. In the first cross (*yC/yc* by *yc*) there is in Jb a 1:1 ratio (35*yC*, 36*yc*), indicating that the X chromosome in all or nearly all males came from the female parent. The difference between these values and the 1:3 ratio expected if selection is at random between a paternal and the maternal X chromosome is highly significant. In Ja the numbers are 6*yC* and 12*yc*. Here there may be paternal X chromosomes altering the ratio, but the numbers are too small to be significant. In the second cross (*YC/yC* by *yc*) one can tell just where each X chromosome came from. In Jb four out of 118 males got the paternal X chromosome and in Ja three out of twelve. When these numbers are set in the two-by-two table it is found that

the difference between Ja and Jb is highly significant, since chi-square is 9.98. Thus Ja and Jb do not represent samples from the same population with regard to the number of males which received the paternal X chromosome. In those considered to be from "unisexual" progenies a significantly larger proportion got it than in those of the "bisexual" progenies. The males of male progenies in group J all received the maternal X chromosome. The males in predominantly female "bisexual" progenies are intermediate between exceptional males and normal males in that their proportion in the progeny is greater than that of exceptional males but not so great as that of normal males, which occur alone or with but a few females. It seems that where there is a tendency for exceptional males to inherit the paternal X chromosome; this tendency is less marked in closely related males of predominantly female "bisexual" progenies.

In males of male progenies the inheritance of the X chromosome has been regularly from the female parent. In more than five thousand cases from the crosses Y/Y by y and y/y by Y only one exception has been found, and this may have been an error.

Thus normal males consistently inherit the maternal X chromosome, while in the case of exceptional males there seems to be in different groups of closely related males various tendencies to inherit one of the paternal X chromosomes. The evidence at hand further indicates that in males of "bisexual" female progenies the tendency to inherit a paternal X chromsome is not so great as in closely related exceptional males.

"Bisexual" and "unisexual" strains of *S. ocellaris* are interfertile. As has been mentioned, a great variety of ratios of males to females may be obtained in different progenies. It may vary from one extreme to the other—from all males to equal numbers of males and females to all females. In predominantly female "bisexual" progenies the ratio may be significantly different from 1:1 and still contain a considerable number of males. It appears that in such cases the predisposition toward femaleness is not so great as in progenies which have only a few exceptional males. Irregularities in the inheritance of the X chromosome are common among exceptional males but do not apear to occur among normal males. These irregularities seem to be associated with something which causes the elimination of two X chromosomes instead of one from the soma in the presence of a predisposition toward femaleness (toward the elimination of one instead of two), but not associated with it in the absence of this predisposition toward femaleness. When the predisposition toward femaleness is less—that is, in "bisexual" progenies—the number of irregularities might, on this basis, be expected to be smaller. The evidence from group J indicates that this is so.

Twenty-four exceptional males which had received their X chromosome from the male parent were mated, many of them with six females each. All were sterile though they mated readily. An unrecorded number of these were dissected, and all had rudimentary gonads with no sperms in them or in their ducts. Several hundred similar dissections were made on normal males, and though variation was found, the gonads generally were large and they and

their ducts were filled with sperms. The rudimentary gonads of the sterile males were lumpy in form. Some of the gonads of normal males had at their distal ends masses of clear, lumpy material similar to the gonads of the sterile males. Gonads were removed from an unrecorded number of exceptional males which got their X chromosome from the female parent. All were found to be smaller than the average size of those from normal males. One such pair of gonads which were removed after the male had mated were about the size and condition of those from a sterile male of the paternal X chromosome type, but there were sperms in the duct. This male proved to be fertile. Others were somewhat larger and contained sperms but with much of their bulk made up of the clear, lumpy material characteristic of the sterile ones.

From these observations it appears that the sterility is caused by the lack of sperms and not because of abnormal chromosome numbers in the sperms or failure of the males to mate. It is not known what kind of chromosome elimination takes place in the germ line of these males. Since it is abnormal in the soma it may be abnormal in the germ line also. Normally in both males and females one paternal X chromosome is eliminated from the germ line during development, leaving the other paternal one and the maternal one. In the first meiotic division of spermatogenesis a mono-polar spindle is formed, and all the chromosomes of maternal origin go to the pole, and those of paternal origin go away from it and are budded off. If the maternal X chromosome is eliminated from the germ line as well as from the soma and at the first meiotic division the paternal chromosomes go away from the pole and are lost, there would be no X chromosome left, and spermatogenesis might be unable to continue without one. This, however, would not explain the condition in exceptional males which got their somatic X chromosome from the female parent and which have small abnormal gonads but are fertile. Exceptional males may be considered males which should have been females. The factor which is in the eggs of a female-producing female which tends toward the elimination of only one X chromosome instead of two—a tendency toward femaleness—may persist in the exceptional males and interfere with spermatogenesis. It would be necessary to assume further that this environment is less compatible to an X chromosome from the male parent than to a maternal one. It would be well to study the gonads of males from predominantly female "bisexual" progenies to see if they are intermediate between those of normal and of exceptional males and to see if a correlation exists between the size of the lumpy mass on fertile gonads and the sex ratio of the progeny. This work has been interrupted by the war.

SUMMARY

Males of male progenies regularly inherit the maternal X chromosome, only one possible exception being observed in more than five thousand cases. Among exceptional males there may be a strong tendency toward the inheritance of the maternal or of a paternal X chromosome or there may be a random selection between the maternal and a paternal X chromosome. In the cases observed there was consistency in the type of selection within each group made

up of the offspring of sibs. There is evidence that in males of predominantly female "bisexual" progenies the amount of inheritance of a paternal X chromosome is less than that in closely related exceptional males. Exceptional males which inherit an X chromosome from the male parent are sterile.

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GENETIC VARIATION IN THE CLOVER LEAFHOPPER'S ABILITY TO TRANSMIT POTATO YELLOW-DWARF VIRUS

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INTRODUCTION

STOREY (1932) discovered that individuals of *Cicadulina mbila* (Naude) varied in ability to transmit maize-streak virus, *Marmor maidis* var. *typicum* H. (HOLMES 1939). He was able to breed two races, an active race that could transmit the virus and an inactive race that was unable to do so. His experiments indicated that ability to transmit was controlled by a single sex-linked dominant gene. Later, BENNETT and WALLACE (1938) showed that families of *Eutettix tenellus* (Baker) vary in their ability to transmit curly-top virus (*Chlorogenus eutettícola* H.).

It is the purpose of this paper to deal with the hereditary variation in transmission of potato yellow-dwarf virus, *Marmor vastans* H. var. *vulgare* Black (BLACK 1940) by individual leafhoppers of the species *Aceratagallia sanguinolenta* (Prov.). The different expressions of this genetic variability are in part elucidated, and it is shown that by selective breeding, races may be obtained that differ greatly in their infective ability. An abstract of this work has already appeared (BLACK 1941).

MATERIALS AND METHODS

Materials and methods of general application in the present work will be described here; any deviations in individual experiments will be dealt with under such experiments. Crimson clover (*Trifolium incarnatum* L.), reported earlier (BLACK 1938) as susceptible to the virus, was selected as a test plant. It shows distinctive symptoms when infected with yellow dwarf and has certain advantages over potato (*Solanum tuberosum* L.) as a test plant in insect transmission studies. It is more readily grown from seed to obtain virus-free seedlings; the plants are better hosts for the leafhopper; the seedlings are readily accommodated in smaller cages and for a longer period. The crimson clover seed was sown in sand and the young seedlings transplanted individually to soil in 2½-inch pots shortly after expansion of the unifoliate leaf. Sowings were timed so that seedlings with the first trifoliate leaf just beginning to unfold were available when required for testing the infectivity of insects. Each seedling was covered by a celluloid cylinder having a cheesecloth top and a small hole cut in one side near the upper end. The insect to be tested was introduced through the hole and the aperture plugged with nonabsorbent cotton. The insects were in the nymphal stage when placed on their first test plants, and since they were tested individually, they were unable to mate and reproduce when they reached the adult stage. Subsequent transfers were made by tilting the pot and cage at about a 45° angle toward a good source of light, in-

ducing the insect to hop to the upper part of the cage, and substituting a new pot and seedling at the bottom. With practice, transfers to fresh plants could be made rapidly in this manner. After the removal of the insect, each test plant was held for observation in the greenhouse for five weeks or longer.

The statistical significance of the data obtained was determined by extensive use of the fourfold table to ascertain the chi-square values. Yates' correction was employed in all cases. From these values the probabilities were determined by referring to tables in FISHER (1936) or YULE (1922). Throughout the paper the expression "odds x:1" indicates the probability that any difference is not due to chance alone.

ACTIVE AND INACTIVE RACES

Sixty clover leafhopper nymphs that had fed for 12 days on infected crimson clover were tested individually for their ability to transmit. Some infected several plants, while others infected none. This result suggested the possibility of obtaining active and inactive races of the clover leafhopper corresponding to those secured by STOREY for *Cicadulina mbila*. Insects transmitting the virus were bred among themselves in an attempt to obtain an active race, while those failing to transmit were also bred among themselves in an attempt to obtain an inactive race. Because of the life history of the insect and the latent period of infection in the plant, it was necessary to make the matings when about half of the results of the infectivity tests were available. Therefore, about four times as many matings were made as were actually needed, and each impregnated female was caged separately on a crimson clover plant in the acute stage of yellow dwarf. By the time the progeny was ready to be tested, all the results of the infectivity tests on the parents were available and it was then possible to select and test the best families and so to continue each line. In general, among the individuals of the active race the male infecting most plants was mated with the corresponding female. Some males were used to impregnate as many as three females. Many brother to sister matings were made in producing the third, fourth, and fifth generations. However, debility due to inbreeding manifested itself in slower growth, infertility, early death, and lack of vigor. Such matings were therefore avoided in later generations. Nevertheless, debility due to inbreeding was a serious handicap, especially in the active race. When the two races were crossed, the increase in the number, vigor, longevity, and growth rate of the progeny was striking.

The breeding program was carried through nine generations, not counting the original parental generation. In each generation an attempt was made to test 105 individuals in each race, 15 individuals in each of seven families. The test of the active and inactive race in each generation was carried out at the same time and under the same conditions, except that each family of insects was fed on a different plant. These plants, however, were selected for uniformity. An endeavor was made to transfer insects that were being tested for infectivity to a fresh plant every week. This was not always possible, but on the average each insect was tested on five plants over a period of six weeks. The number of cast skins, the deaths, and the sex of newly emerged adults

were recorded at each transfer so as to furnish a complete record of each insect. The incubation period in each infected clover plant was also recorded. Examples of the data sheets for a family of the active race and for one of the inactive race are presented in figure 1. Presentation and analysis of some of the data on ecdyses and deaths of the insects and incubation period in the plant are reserved for another paper. The data analyzed in the present paper

Active race, generation 9, family 23.

SEX →		♀	♀	♀	♂	♀	♂	?	♀	♀	♀	♂	♂	♀	♂	♂
INSECT NO. →		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Dates	6-29-39	^{a}	^{o}	^{a}	3^{a}	^{a}	4^{o}	d	3^{a}	^{a}	2^{a}	3^{o}	^{a}	^{a}	^{a}	2^{a}
insects	7- 6-39	d	^{a}	d	3	4	^{a}			5	3	^{a}	pd	d		3
placed on	7-13-39				3				3	3	2		3			2
successive	7-20-39		2		4	2	5		3	2	3		3			2
test plants	7-27-39		2		2	2	1		3	3	1	2	4		2	d

Inactive race, generation 9, family 2.

SEX →		♂	♀	♂	♂	♂	♀	♂	♀	♂	♂	♂	♂	♂	♀	?
INSECT NO. →		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Dates	6-29-39	^{a}	3^{a}	^{a}	^{a}	^{a}	^{o}	^{a}	^{a}	^{a}	^{o}	^{a}	^{a}	^{a}	^{a}	^{o}
insects	7- 6-39		3				^{a}				^{a}					d
placed on	7-13-39		2													
successive	7-20-39		2_{d}													
test plants	7-27-39			d	d	d										

FIGURE 1.—Examples of the records kept on the performance of each family of insects tested. Each number indicates the week in which the crimson clover test plant showed the first symptoms of yellow dwarf. (Weeks were counted from the day the insect was removed from the plant.) Superscript ^{o}=no cast skin present when insect removed from plant; superscript ^{o}=a cast skin found, insect still in nymphal stage; superscript ^{a}=cast skin found, insect now an adult. Subscript $_{d}$=insect dead when removed from plant; pd=plant died from causes other than yellow dwarf.

are restricted to those insects which survived at least the period during which they were confined to the first test plant. The data are also restricted to those plants from which the insects were removed alive. For comparisons involving sex it was necessary to eliminate also all data appertaining to insects that died as nymphs.

Members of the active selection fed on 3340 plants and infected 1845, or 55.24 percent, whereas members of the inactive race fed on 3762 and infected 204, or 5.42 percent. It is apparent from table 1 that in each generation there was a striking difference in the percentage of infective insects in the two races. In every case the odds against the difference being due to chance alone were greater than 100:1. In the ninth generation the active race contained 80 per-

TABLE 1

Genetic variation in the ability of the clover leafhopper to transmit potato yellow-dwarf virus.

GENERATION	ACTIVE RACE		INACTIVE RACE	
	INSECTS INFECTIVE / INSECTS TESTED	PERCENTAGE OF INFECTIVE INSECTS	INSECTS INFECTIVE / INSECTS TESTED	PERCENTAGE OF INFECTIVE INSECTS
1	53/60	88.3	27/60	45.0
2	16/39	41.0	4/44	9.1
3	78/101	77.2	20/103	19.4
4	67/103	65.0	1/97	1.0
5	87/92	94.6	6/99	6.1
6	72/91	79.1	4/102	3.9
7	84/92	91.3	17/103	16.5
8	71/94	75.5	2/104	2.0
9	78/98	79.6	2/104	2.0
Total	606/760	79.7	83/816	10.2

cent of infective insects, while the inactive race contained only 2 percent.[1] It is of interest to note that after this program of selective breeding the inactive race should still contain active individuals, for in the nine preceding generations all their direct ancestors had failed to transmit the virus. Moreover, beginning with the breeding of the seventh generation it was possible to continue the inactive selection by mating only individuals from families, none of whose 15 members transmitted the virus. In this connection it is worth noting that by the second generation the inactive selection was confined to the descendants of only four of the original insects. If multiple factors are involved in the ability to transmit, it might be that the breeding program as carried out produced the least active race possible from the recombination, without

[1] In spite of the failure to isolate a race none of whose members would transmit, it is thought best to retain STOREY's terminology "active" and "inactive" to designate the races.

mutation, of genes present in these four insects. Inclusion of additional insects might make possible the selection of an even less active race. Similar considerations may be involved in the failure to get 100 percent of the members of the active race to transmit in spite of the fact that for nine generations all the ancestors of the race had transmitted the virus.

HYBRIDS BETWEEN THE TWO RACES

Hybrids between the two races were obtained by mating individuals belonging to the seventh and eighth generations. Tests of these hybrids were carried out at the same time and under the same conditions as tests of the eighth and ninth generations, respectively. In the eighth generation, out of 104 hybrids tested, 22, or 21.2 percent, were infective, and in the ninth generation, out of 102 hybrids tested, 31, or 30.4 percent, were infective. In each generation the odds are greater than 100:1 that the difference between the hybrids and either race is not due to chance alone. It is clear that the hybrids are intermediate in their ability to transmit. The fact that the vigor of the hybrids was noticeably greater than that of either race is considered good evidence that the differences in transmission by the active and inactive races and by the hybrids are not correlated with vigor.

SEX-LINKAGE

In table 2, the data on the infectivity of the hybrids of the eighth and ninth generations are presented in detail. The outstanding feature of the table is the high proportion of infective males to noninfective males (26:33) resulting from crosses between inactive males and active females. This proportion is significantly different (odds greater than 100:1) from the proportions in groups a or b or a+b+d (table 2). However, the odds are only 16:1 that the differ-

TABLE 2

Active and inactive progeny of crosses between the two races of clover leafhoppers.

CULTURE	PARENTS: ACTIVE ♂ × INACTIVE ♀				CULTURE	PARENTS: INACTIVE ♂ × ACTIVE ♀			
	MALES (GROUP A)		FEMALES (GROUP B)			MALES (GROUP C)		FEMALES (GROUP D)	
	ACTIVE	INACTIVE	ACTIVE	INACTIVE		ACTIVE	INACTIVE	ACTIVE	INACTIVE
G8-19	1	3	4	7	G8-25	3	9	0	1
G8-20	0	8	0	7	G8-27	2	5	0	7
G8-22	1	9	1	4	G8-28	5	3	3	3
G9-34	1	7	0	7	G8-29	2	5	0	9
G9-36	1	6	1	7	G9-39	7	3	4	1
G9-37	4	2	3	5	G9-40	6	2	2	5
G9-38	0	7	0	7	G9-41	1	6	1	6
Total	8	42	9	44	Total	26	33	10	32

ence between the proportions in groups c and d is not due to chance and group d is the closest control on group c, since the insects in c and d were from the same families and within each family fed on the same source plants. Differences between a, b, and d are not significant (odds 1:1 or less).

If we consider the data on numbers of plants infected by the hybrids (table 3), we find that in group c the proportion of plants infected to plants healthy is significantly different (odds greater than 100:1) from the proportions in groups a, b, or d. Differences between a, b, and d are not significant (odds less

TABLE 3

Condition of plants inoculated by progeny of crosses between the two races.

CULTURE	PARENTS: ACTIVE ♂ × INACTIVE ♀				CULTURE	PARENTS: INACTIVE ♂ × ACTIVE ♀			
	MALES (GROUP A)		FEMALES (GROUP B)			MALES (GROUP C)		FEMALES (GROUP D)	
	PLANTS INFECTED	PLANTS HEALTHY	PLANTS INFECTED	PLANTS HEALTHY		PLANTS INFECTED	PLANTS HEALTHY	PLANTS INFECTED	PLANTS HEALTHY
G8-19	1	15	7	36	G8-25	7	41	0	4
G8-20	0	32	0	27	G8-27	2	26	0	28
G8-22	2	38	2	18	G8-28	6	26	4	20
G9-34	3	37	0	35	G8-29	1	25	0	36
G9-36	3	32	3	37	G9-39	30	20	10	13
G9-37	14	16	13	26	G9-40	23	17	2	33
G9-38	0	35	0	35	G9-41	4	30	1	31
Total	23	205	25	214	Total	73	185	17	165

than 1:1). In view of these various comparisons, there seems to be good reason for concluding that males produced by mating inactive males and active females are significantly different, in regard to ability to transmit, from the females produced by such crosses and from both males and females resulting from the reciprocal crosses. These results suggest that sex-linkage is involved in the ability to transmit or that an autosomal gene (or genes) with a maternal (plasmatic) effect has a different degree of dominance in the two sexes. These two hypotheses will be discussed later.

PERCENTAGE OF INFECTIVE MALES AND FEMALES

In table 4, data on the infective males and females in different population groups are presented. In both races and in the hybrids between them there is a higher percentage of infective males than females. In the active selection and in the population as a whole, the odds are greater than 20:1 that this difference is not due to chance alone. Since these populations are of mixed genetic constitution, the greater transmission by the males probably has the same basis as the results obtained with the hybrids alone.

TABLE 4

Percentage of male and female leafhoppers transmitting the potato yellow-dwarf virus.

INSECT GROUP	SEX	TOTAL INSECTS	INFECTIVE INSECTS	PERCENTAGE OF INFECTIVE INSECTS	ODDS THAT DIFFERENCE IS NOT DUE TO CHANCE ALONE
Active	Male	352	285	81.0	25:1
	Female	373	277	74.3	
Hybrids	Male	109	34	31.2	10:1
	Female	95	19	20.0	
Inactive	Male	379	45	11.9	7:1
	Female	397	33	8.3	
All insects	Male	840	364	43.3	32:1
	Female	865	329	38.0	

EFFICIENCY OF TRANSMISSION

The number of plants infected divided by the number of plants on which infective insects were tested has been employed as a measure of the efficiency of transmission. For convenience, the resulting fraction has been termed the efficiency index. It should be emphasized here that noninfective insects and the plants on which they were tested do not enter into the calculation of the efficiency indices. For example, most of the members of the inactive race and the plants on which they were tested are not considered because most of the insects were noninfective. The efficiency index for the inactive race was derived from only the small fraction of insects in this race that were infective and the plants on which these infective insects were tested.

TABLE 5

Efficiency of transmission of potato yellow-dwarf virus by male and female leafhoppers.

INSECT GROUP	SEX	PLANTS INOCULATED	PLANTS INFECTED	EFFICIENCY INDEX	ODDS THAT DIFFERENCE IS NOT DUE TO CHANCE ALONE
Active race	Male	1285	901	0.70	13:1
	Female	1256	838	0.67	
Hybrids	Male	152	96	0.63	9:1
	Female	82	42	0.51	
Inactive race	Male	221	130	0.59	97:1
	Female	156	70	0.45	
All insects	Male	1658	1127	0.68	92:1
	Female	1494	950	0.64	

The efficiency indices for infective males and females (table 5) in the different population groups are consistently higher for the males. The difference is slight and is significant only in the inactive race and in the total population. In table 6 the data are rearranged to show the differences in the efficiency among the infective members of the active and inactive races and the hybrids. Insects in the active race were significantly more efficient than insects of the same sex in the inactive race. In both sexes the efficiency of the hybrids fell between that of the two races. However, the only significant differences were

TABLE 6

Efficiency of transmission of potato yellow-dwarf virus in the two races of leafhoppers and their hybrids.

SEX	INSECT GROUP	PLANTS INOCULATED	PLANTS INFECTED	EFFICIENCY INDEX	ODDS THAT DIFFERENCE IS NOT DUE TO CHANCE ALONE	
Male	Active	1285	901	0.70	10:1	>100:1
	Hybrid	152	96	0.63		
	Inactive	221	130	0.59	1:1	
Female	Active	1256	838	0.67	>100:1	>100:1
	Hybrid	82	42	0.51		
	Inactive	156	70	0.45	1:1	
Both sexes	Active	2541	1739	0.68	>100:1	>100:1
	Hybrid	234	138	0.59		
	Inactive	377	200	0.53	5:1	

>=greater than.

those between female hybrids and female members of the active race and between the totals for the active race and the hybrids. In general, it appears that greater efficiency and a higher proportion of infective insects are correlated whether the latter proportion is associated with the race or the sex.

DISCUSSION

The genetics of the ability of the clover leafhopper to transmit potato yellow-dwarf virus is complicated by the fact that even under favorable experimental conditions one cannot be certain that an insect which possesses the ability to transmit will do so. That insects having this ability failed to transmit is indicated by the fluctuations in the percentage of infective insects from generation to generation (table 1). A small percentage of infective insects did not infect any but the last plant on which they were tested. This suggests that,

had the insects been transferred to additional plants, a few more might have been added to the infective class.

It is possible that an autosomal gene or genes in the homozygous condition might reduce the chances of an insect becoming infective to the low level existing in the inactive race, might reduce the efficiency of the few infective insects in this race, and, in the heterozygous condition, might exert an intermediate action both on the probability of an insect becoming infective and on efficiency. If in addition the gene, or genes, produced a maternal (plasmatic) effect on the egg cytoplasm with a different degree of dominance in the two sexes, all the data might be accounted for.

A simpler explanation must also be considered—namely, that the sex mechanism is the same as in Drosophila and that a single incompletely dominant gene affecting the tendency of an insect to transmit and the efficiency of transmission is located on the sex chromosome. Such an arrangement accounts for the proportion of infective insects among the hybrids of groups a, b, and d of table 2 being lower than that of group c. However, it does not account for the percentage (44.1 percent) of infective insects in group c being significantly less (odds greater than 100:1) than the percentage (77.6 percent) of infective insects in the active race during the eighth and ninth generations. If the hypothesis under consideration were true, there should be no difference between the insects in group c and those of the eighth and ninth generations of the active race. It is suggested that the difference may be due to other inhibiting genes present on other chromosomes.

It is worth pointing out here that the active and inactive groups in hybrids between the active and inactive races of *Aceratagallia sanguinolenta* are different from the corresponding groups in *Cicadulina mbila*. In *A. sanguinolenta* group c (table 2) might be considered active and groups a, b, and d inactive. In *C. mbila* (STOREY 1932) the group corresponding to a is inactive and the groups corresponding to b, c, and d are active.

SUMMARY

By selective breeding through ten generations, two races of clover leafhoppers have been obtained, one "active," the other "inactive." In the last generation, 80 percent of the active race, 2 percent of the inactive race, and 30 percent of the hybrids proved infective when tested under the same conditions. Infective individuals appeared in every generation in the inactive race, even though all ancestors in preceding generations had failed to transmit the virus. Conversely, in every generation some individuals in the active race failed to transmit the virus, although all ancestors in preceding generations had transmitted.

A slightly but significantly higher percentage of males (43 percent) than of females (38 percent) transmitted the virus. Although a difference between males and females was demonstrated to be significant only in the total population and in the active race, the difference was in the same direction in the inactive race and in the hybrids.

The male infective insects were more efficient vectors than the female infective insects in both races and in the hybrids. However, such differences in efficiency were demonstrated to be significant only for the whole population and for the inactive race. Infective members of the active race were significantly more efficient vectors than infective individuals of the same sex belonging to the inactive race. The hybrids were intermediate in efficiency, but in most cases their position was not demonstrated to be significant.

Evidence was obtained indicating that the males resulting from crosses between inactive males and active females were more active than the females derived from such crosses and more active than the males and females resulting from reciprocal crosses. Two genetic hypotheses explaining these differences are discussed.

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A CYTOGENETIC STUDY OF THE YELLOW-SCUTE REGION OF THE X CHROMOSOME IN DROSOPHILA MELANOGASTER

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INTRODUCTION

A DETAILED study of the *y-sc* region in *Drosophila melanogaster* has been made by means of an analysis of a series of changes in the loci yellow (*y*), achaete (*ac*), and scute (*sc*), which are included in that region.

The purposes of this study were (a) to determine the bands of the salivary gland chromosomes with which these loci are associated, (b) to find out the relationship between cytological and genetical changes in this region, and (c) to compare the cytogenetic findings for the *y* and *sc* loci with those for other loci in the X chromosome.

These points will be discussed after the presentation of a preliminary description of the changes studied.

MATERIALS AND METHODS

With a few exceptions, noted in the descriptions, the changes were obtained by irradiating wild type (Swedish-b) males with X-rays at a dosage of 2,500–3,000 roentgen units and picking up induced changes in the *y* and *sc* loci among the F_1 females from the cross *y sc* or *y sc w* females × irradiated males.

The *y* or *sc* mutant females thus obtained were mated with *y Hw w* males, and kept in balanced stocks.

Slides were made from the salivary glands of heterozygous female larvae of the constitution mutant/*y Hw w*, and were stained either with aceto-carmine or with acetic orcein.

The equipment used in analyzing these slides consisted of a 90X, 1.3 N.A. apochromatic objective, with an oil immersed 1.4 N.A. condenser, 12.5X compensating oculars, and a Bausch and Lomb research lamp with the green Wratton filter number 61.

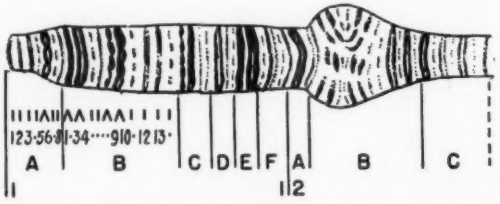

FIGURE 1.—Diagram of left end of the salivary gland X chromosome, with bands numbered after BRIDGES' (1938) map.

DESCRIPTION OF CHANGES

The collection of changes studied can be divided into three groups, as follows: I. Genetic changes not associated with chromosomal aberrations (seven cases). II. Genetic changes associated with chromosomal aberrations (19 cases). III. Cytological changes without genetic effect (two cases).

The cytological analyses are based on BRIDGES' (1935, 1938) maps of the salivary gland chromosomes. The *y-sc* region of the X chromosome is shown in figure 1. Some of the changes included in this collection were analyzed and published previously by DEMEREC and HOOVER (1936a).

I. GENETIC CHANGES WITHOUT CYTOLOGICAL ABNORMALITY

(*a*) *Yellow locus*

1. y^{260-4} (yellow 260-4) Found by DEMEREC 1938, unpublished. From X-rayed *sn B* male. Not lethal. Phenotypically like y^2; *sc* not affected.
2. y^{260-12} (yellow 260-12) Found by SUTTON 1939, unpublished. From X-rayed Sw-b male. Not lethal. Phenotypically like y^1; *ac* and *sc* not affected.
3. y^{260-24} (yellow 260-24) Found by SUTTON 1939, unpublished. From X-rayed Sw-b male. Not lethal. Phenotypically like y^1; *ac* and *sc* not affected.
4. y^{260-28} (yellow 260-28) Found by SUTTON 1939, unpublished. From X-rayed Sw-b male. Viability of males reduced. Phenotypically like y^1; *ac* also affected, but not *sc* or *svr* (silver).
5. y^{260-30} (yellow 260-30) Found by BISHOP 1940, unpublished. From X-rayed Sw-b male. Not lethal. Phenotypically like y^1; *ac*, *sc* and *svr* not affected, but *f* (forked) also affected.
6. y^{260-31} (yellow 260-31) Found by FANO 1941, unpublished. From X-rayed Sw-b male (12,000 r). Lethal in homozygous and hemizygous condition. y^{260-31}/y^1 phenotypically like y^1; *ac* also affected. Salivaries show a piece of IIL (24-29) inserted into X about 9A. Aberration independent of change at *y* locus. Genetic tests show that y^{260-31} does not cover the recessive lethal associated with Df 260-25, therefore the recessive lethal in y^{260-31} is associated with the change at the *y* locus and not with the rearrangement.

(*b*) *Scute locus*

1. sc^{260-16} (scute 260-16) Found by SUTTON 1938, unpublished. From X-rayed Sw-b male. Lethal in homozygous and hemizygous condition. *sc* does not always show in sc^{260-16}/sc females; *y* not affected.

II. GENETIC CHANGES ASSOCIATED WITH CHROMOSOMAL ABERRATION

(*a*) *Yellow locus*

1. y^{260-2} (yellow 260-2) DEMEREC and HOOVER 1936 (published as Deficiency 260-2). From X-rayed Sw-b male. Lethal in homozygous and hemizygous condition. y^{260-2}/y^1 phenotypically like y^1; *y* and *ac* affected, but not *sc*. Deficiency for bands 1A1 to 1B1.2 inclusive.

2. y^{260-10} (yellow 260-10) SUTTON 1940 (published as Df (1) 260-10). From X-rayed Sw-b male. Not lethal. Males and homozygous females phenotypically like y^1; also achaete. *y* and *ac* affected, but not *sc*. Deficiency for bands 1A1, 2.

3. y^{260-11} (yellow 260-11) Found by SUTTON 1939, unpublished. From X-rayed Sw-b male. Not lethal. Males sterile. Males and homozygous females phenotypically like y^1; *y* affected but not *ac* and *sc*. T(X; IIIR). Break in X between 1B1.2 and 3.4, in IIIR between 85F1 and 5.

4. y^{260-13} (yellow 260-13) Found by SUTTON 1939, unpublished. From X-rayed Sw-b male. Not lethal, fertility of males reduced. Body wild type, bristles yellow in males and homozygous females. *ac* and *sc* not affected. T(X; IIL). Break in X between 1A4 and 5.6, in IIL at 36D. Stock lost.

5. y^{260-21} (yellow 260-21) Found by SUTTON 1939, unpublished. From X-rayed Sw-b male. Lethal in homozygous and hemizygous condition. y^{260-21}/y^1 phenotypically like y^1; *sc* not affected; not tested for *ac*. Inversion, with breaks between 1A5.6 and 7 and between 5D7.8 and E1.2. T(X; IIIL) also present, with break in X at 6C and in IIIL at 70E-F.

(*b*) *Scute locus*

1. sc^{260-14} (scute 260-14) Found by SUTTON 1939, unpublished. From X-rayed Sw-b male. Not lethal. *sc* affected, but not *y*, *ac*, or *svr*. Males and homozygous females scute. Inversion, with breaks between 1B1.2 and 3.4 and between 11D3 and 8.

2. sc^{260-15} (scute 260-15) Found by DEMEREC 1938, unpublished. From X-rayed Sw-b male. Viability reduced. Males sterile. *sc* affected, but not *y* or *ac*. Males and homozygous females scute. T(X; IIIL). Break in X between 1B3.4 and 5, in IIIL between 71C and D.

3. sc^{260-17} (scute 260-17) Found by SUTTON 1939, unpublished. From X-rayed Sw-b male. Not lethal. *sc* affected, but not *y*, *ac* or *svr*. Males and homozygous females scute. T(X; IIL). Break in X between 1B1.2 and 3.4, in IIL about 31C.

4. sc^{260-18} (scute 260-18) Found by SUTTON 1939, unpublished. From X-rayed Sw-b male. Not lethal. Males sterile. Males and homozygous females scute. *sc* affected, but not *y*, *ac* or *svr*. T(X; IIR; IIIL). Breaks in X between 1A5-6 and 1B1.2 and between 7A1.2 and B1.2; in IIR chromocenter between 41D and E; and in IIIL chromocenter at 80C. New order: Tip of X-1A5.6/41D-sp.a. II-21 (Tip of IIL); tip of IIR-41E /1B1.2-7A/80C-sp.a. III-tip of IIIR; tip of IIIL-80C/7B-sp.a. X. (sp.a. = spindle attachment.)

5. sc^{260-20} (scute 260-20) Found by SUTTON 1939, unpublished. From X-rayed Sw-b male. Not lethal. *sc* affected, but not *y*, *ac* or *svr*. Males and homozygous females scute. T(X; IIIL). Break in X between 1A8 and 1B1.2, in IIIL preceding 61A1.2, at extreme tip.

6. sc^{260-22} (scute 260-22) Found by SUTTON 1939, unpublished. From X-rayed Sw-b male. Not lethal. *sc* affected, but not *y*, *ac* or *svr*. Males and homozygous females scute. Inversion with breaks between 1B1.2 and 3.4 and between 1E1.2 and 3.4.
7. sc^{260-23} (scute 260-23) Found by SUTTON 1939, unpublished. From X-rayed Sw-b male. Not lethal. *sc* affected, but not *y* or *svr; ac* not adequately tested. Males and homozygous females scute, apparently not achaete. T(X; ?). Break in X between 1B1.2 and 3.4. Stock lost.
8. sc^{260-26} (scute 260-26) Found by SUTTON 1939, unpublished. From X-rayed Sw-b male. Viability of males reduced, fertile. *sc* affected, but not *y*, *ac* or *svr*. T(X; IIL; IIR). Break in X between 1B3.4 and 5; in IIL between 27D1.2 and 3; and in IIR at 41A, 41F and 58B. New order: tip of X-1B3.4/41F3-58B/1B5-sp.a. X; tip of IIR-58B/41F-41A/27D-sp.a. II-40/27D-tip of IIL.
9. sc^{260-27} (scute 260-27) Found by SUTTON 1939, unpublished. From X-rayed Sw-b male. Males sterile, with reduced viability. *sc* affected, but not *y*, *ac* or *svr*. Males and homozygous females scute. T(X; IIL; IIR). Breaks in X between 1A8 and 1B1.2, at 15E and at 19F (chromocenter); in IIL at 33-34; and in IIR at 57B-C. New order: Tip of X-1A8/20A-sp.a. X; tip of IIL-33/15E-19F/1B1.2-15E/57B-sp.a. II-34/57-tip of IIR.
10. sc^{260-29} (scute 260-29) Found by SUTTON 1940, unpublished. From X-rayed Sw-b male. Not lethal, males sterile. *sc* affected, but not *y*, *ac* or *svr*. Males and homozygous females scute. T(X; IIL; IIIL). Break in X between 1A5.6 and 1B1.2; in IIL between 22A and B, and between 34A and B; and in IIIL between 75C and E. New order: Tip of X-1A8/34A-22B/34B-sp.a. II-tip of IIR; tip of IIL-22A/75E-sp.a. III-tip of IIIR; tip of IIIL-75C/1B1.2-sp.a.X.
11. *l*(1) 272-13 (lethal (1) 272-13). Found by DEMEREC 1940, unpublished. From X-rayed *y* male. Lethal in homozygous and hemizygous condition. *l*(1)272-13/*sc* phenotypically scute. *sc* affected, but not *y*, *ac*, *svr*. Complex rearrangement of X, with four breaks: first between 1A5.6 and B1.2; second between 11A6.7 and 8.9; third between 11F1.2 and 12A1.2; fourth between 18A3.4 and B1.2. New order: Tip of X-1A5.6/12A1.2-18A3.4/11A6.7-1B1.2/11A8.9-11F1.2/18B1.2-sp.a.X.

(*c*) *Yellow and scute loci*

1. Df(1)*svr* LVM (Deficiency (1) silver L. V. Morgan). Lethal in homozygous and hemizygous condition. *y*, *ac*, *sc* and *svr* affected. Females heterozygous for Df(1) *svr* L.V.M. and for *y*, *ac*, *sc* or *svr* show the recessive character. Deficiency for bands 1A1-1B9.10 inclusive (presence of 1B11, 12 uncertain).
2. Df(1)260-1 (Deficiency (1)260-1) DEMEREC and HOOVER 1936. Lethal in homozygous and hemizygous condition. *y*, *ac* and *sc* affected, but not *svr*. Females heterozygous for Df(1)260-1 and for *y*, *ac*, or *sc* show the recessive character. Deficiency for bands 1A1-1B3.4 inclusive.

3. Tp(1)260-25 (Transposition (1)260-25) SUTTON 1940. From X-rayed Sw-b male. Lethal in homozygous and hemizygous condition. Extreme *sc*, shows variegation for *y* and *ac*. Tp(1)260-25/*sc* females are scute, Tp(1)260-25/*y ac* females show variegation for *y* and *ac*. Transposition; terminal bands 1A1-1B1.2 transferred to X chromocenter. The deficiency for these terminal bands obtained by crossing-over was published as Df(1)260-25 (SUTTON 1940).

III. CYTOLOGICAL CHANGE WITHOUT GENETIC EFFECT

1. Df(1)260-5 (Deficiency (1)260-5) DEMEREC and HOOVER 1936a. Detected cytologically in salivary gland chromosomes of an induced *ct* (cut) stock. Not lethal. *y*, *ac* and *sc* not affected. Males and homozygous females wild type. Deficiency for bands 1A1-4 inclusive.

2. Df(1)260-19 (Deficiency (1)260-19) SUTTON 1940. Detected cytologically in salivary gland chromosomes of a stock of Stern (yy/g^2B). Not lethal. *y*, *ac* and *sc* not affected. Males and homozygous females wild-type. Deficiency for bands 1A1, 2.

DISCUSSION

Location of genes in the salivary gland chromosomes

The yellow and achaete loci

In Df(1)260-5, bands 1A1-4 are lost, but the *y*, *ac* and *sc* loci are unaffected. The conclusion can therefore be drawn that all of these loci lie to the right of 1A4, in spite of the fact that in y^{260-10} yellow seems to be affected by the loss of only 2 bands, 1A1, 2 (see below). From each of the stocks sc^{260-20} and sc^{260-27} it was possible to derive a stock hyperploid for the bands 1A1-8, but not for the bands immediately to the right of 1A8. By mating hyperploid females with *y ac* males and backcrossing the hyperploid F_1 females with *y ac* males, it was shown that the duplication for bands 1A1-8 covers the *y* and *ac* loci. Both of these loci must therefore lie within the region 1A5-8. This means that Hairy wing (*Hw*), which is associated with a duplication of 1B1.2 (DEMEREC and HOOVER 1939), is not a duplication of the *ac* locus, as was previously suggested.

The scute locus

In Df(1)260-2 the *sc* locus is unaffected, although bands 1A1-1B1.2 have been lost in this stock. The *sc* locus must therefore lie to the right of 1B1.2. In all the gross rearrangements in which *sc* is affected, the break in this region is either between 1A8 and 1B1.2, between 1B1.2 and 3.4, or between 1B3.4 and 5. No *sc* change was obtained in which the break was further to the right than 1B3.4–5. Since the breaks are clustered around 1B1.2 and 3.4, and since 1B1.2 is excluded by the evidence of Df(1)260-2, 1B3.4 is indicated as the position of the *sc* locus.

"Point mutations" and "position effects" at the yellow and scute loci

Table 1 shows the frequency of mutations associated with different types of rearrangement at the loci of *y* and *sc*. The data for *y* and *sc* are taken from this paper and comparable data for other loci have been provided by DEMEREC and HOOVER (1936b), DEMEREC, KAUFMANN, and SUTTON (1939), and DEMEREC (1940).

In six of the 14 *y* changes, no detectable aberration was found. Three of the remaining eight *y* changes were due to actual deficiency for the *y* locus, and one was associated with a deficiency which did not include the *y* locus, while four were due to gross rearrangements. On the other hand, only one of the 15 *sc* changes showed no aberration, and 12 were due to gross rearrangements or "position effects."

Thus while "point mutations" and changes due to aberrations occurred with about equal frequency at the *y* locus, the *sc* locus behaved as if it were rela-

TABLE 1

Frequency of different types of cytological change associated with genetic changes at the yellow and scute loci.

	y	*sc*
No aberration	6	1
Deficiency	4(3)	2
Rearrangement—with euchromatin	3	7
—with heterochromatin	1	5
Total	14	15

tively stable to "intragenic" changes and relatively susceptible to "position effects." The *sc* locus appears to be more extreme than the *ct* (cut) locus in this respect, and much more so than the *N* (Notch) locus. The yellow phenotype (and scute) can be produced by a point mutation, a deficiency for the locus, or a position effect, all three types being represented in the material under review. The stock y^{260-10} requires some consideration from this point of view, since the change from y^+ to *y* is associated with a deficiency for only two bands, 1A1 and 2. It has been shown above that the *y* locus lies to the right of 1A1, 2, in the region 1A5-8. The yellow phenotype in y^{260-10} therefore cannot be due to deficiency for the *y* locus.

It is difficult to regard this case as being due to a "position effect" caused by the absence of neighboring genes in 1A1, 2, because a similar deficiency (Df (1) 260-19) has no effect on the y^+ phenotype. In spite of the difficulties of cytological analysis in such cases (in relatively unstretched chromosomes, and when the two homologues are superimposed, the deficiency cannot be seen), the author believes that these are both genuine deficiencies, and not the pseudo-deficiencies described by GOLDSCHMIDT and KODANI (1943). They appear consistently the same in all cells where the tips of the two homologues are

well spread and stretched. The analysis was made from a study of several pairs of glands from larvae of the constitutions y^{260-10}/y *Hw w* and Df (1) 260-19/+. The change at the *y* locus in y^{260-10} must therefore be attributed to a point mutation, occurring in one of two ways. (1) The deficiency and the change at the *y* locus may have been quite independent of one another. (2) They may both be results of a single event caused by irradiation. The latter possibility must be taken into account since it has been shown (DEMEREC and FANO 1941) that deficiencies covering several bands may be caused by such a single event.

Among the *sc* rearrangements there are four involving heterochromatin and eight involving euchromatin. Heterochromatin is known to affect loci at some distance from the translocation point, but in euchromatic rearrangements the effects are limited to a shorter distance. Of the euchromatic *sc* rearrangements, five out of eight have a break immediately adjacent to the *sc* band, 1B3.4, either to right or left, and the other three have a break removed to the left of 1B3.4 by one band. Not all breaks immediately adjacent to 1B3.4 are effective in producing *sc* changes, however, as evidenced by y^{260-11} in which there is a break between 1B1.2 and 3.4, and the *y* locus, but not the *sc* locus, is affected.

In this sample of *y* and *sc* changes, the effective range for breaks which affect the *y* locus is the region between 1A2 and 1B3.4, and that for the *sc* locus is the region between 1A8 and 1B5. The data for the *ct* and *N* loci show narrow limits for the "sensitive region" similar to the limits for *sc*. A comparison of "sensitive regions" for several different loci has been made by DEMEREC (1940).

MULLER (1935) suggested the possibility that "position effects" were due to interaction between gene products rather than between the neighboring genes themselves. If this were so, the greater extent, for some genes, of the surrounds ing region in which breaks can produce effective changes could be explained on the further assumption that these genes form primary products in higher concentrations than do other genes; and these products therefore diffuse more rapidly away from the center of production, and are thus enabled to come in contact with and interact with the products of other genes beyond the point of breakage. An alternative assumption would be that in the case of these genes the interacting substances are not the immediate products of genes synthesis, but later products in the chain of reactions between the gene and its ultimate phenotypic expression, so that the time interval before their formation allows the intermediate gene products to diffuse over a greater distance.

SUMMARY

A cytogenetic analysis was made of 28 cytological or genetic changes in the distal region of the X chromosome, in which the loci *y*, *ac*, and *sc* are included.

It is shown that the yellow and achaete loci are associated with bands 1A5-8 of the salivary gland X chromosome, while the scute locus is associated with the 1B3.4 band.

The different types of changes at the *y* and *sc* loci are discussed.

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STUDIES ON THE CREEPER FOWL. XV. MATERNAL INHERITANCE IN SURVIVAL OF EMBRYOS FROM RECIPROCAL CROSSES INVOLVING THE CREEPER FACTOR

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INTRODUCTION

THE Creeper mutation of fowl modifies the morphological and histological structure of the skeleton, especially that of the long bones of the extremities. These skeletal Creeper traits are transmitted as an autosomal dominant. It has been shown previously that the mutation has a twofold effect on embryo viability: it acts as a recessive lethal in the homozygous condition and it reduces the proportion of heterozygous chicks which hatch by approximately five percent below that of normal sibs (LANDAUER and DUNN 1930). A comparison of the results from certain matings suggested the possibility of yet a third effect of the Creeper factor on embryo survival—namely, the existence of differences in viability of embryos in reciprocal crosses of Creeper and normal fowl, the eggs laid by Creeper mothers hatching somewhat less well than those laid by normal hens. Since the data from which this impression was gained were not suitable for a critical test, special matings were arranged, and the present report gives an analysis of the data thus obtained.

MATERIAL

Eggs from reciprocal matings of Creeper and normal chickens were incubated in 1935 and 1936 under standard conditions. Each year Creeper and normal sisters from six to seven Creeper mothers were placed in paired pens and mated with normal and Creeper brothers, respectively. Genotypically, therefore, the eggs in the paired pens were as nearly identical as possible.

The experiment involved two pairs of pens each year, with the matings summarized in table 1. In addition to those listed, the pens contained six normal and four Creeper hens which laid no eggs during the period of the test. In

TABLE 1

Summary of matings and number of birds laying eggs in period of tests.

YEAR	PERIOD	NO. OF SIBSHIPS	CREEPER MALE × NORMAL FEMALES		NORMAL MALE × CREEPER FEMALES	
			PEN NO.	NO. FEMALES	PEN NO.	NO. FEMALES
1935	2/19–6/4	7	13	19	16	22
	2/19–6/4	6	14	19	15	20
1936	4/21–7/14	6	12	20	13	22
	3/ 3–4/14	6	14	16	15	13

1935 a marked increase in the percentage of hatched chicks occurred in all pens in April so that the data for the first eight weeks were analyzed separately from those during the last eight weeks of the observation period. In 1936 the shorter test period in a given pair of pens showed no trend, so that it could be considered as a unit.

The eventual unit in the experiment was the laying hen or sibship which was to be characterized quantitatively in terms of the percentage hatch of its fertile eggs. The Creeper factor did not modify embryo survival so markedly, however, that other genetic and environmental factors could be ignored and considered as part of the experimental error. Seasonal effects that led to a separation of the 1935 data into two periods have already been mentioned. Two other factors were (1) the age at which the embryo died, tested more conveniently with the egg as the unit, and (2) differences between sibships.

TIME OF DEATH OF EMBRYOS

During the 22-day incubation period, mortality was recorded on the seventh, fourteenth, and eighteenth days as well as at the time of hatching. Disregarding differences between sibships and females, all the data from each pen have been summarized in table 2 with respect to the time of death of the embryo. For each pen and developmental period the percentage mortality was based upon the number exposed to death in that interval, subtracting from the total all individuals which had died at an earlier stage. The observed net

TABLE 2

Mortality in relation to age of the embryo for each pen disregarding other differences in parentage.

YEAR	PERIOD	EGGS FROM NORMAL FEMALES						EGGS FROM CREEPER SISTERS					
		PEN NO.	NO. FERTILE EGGS	NET PCT. MORTALITY ON DAYS				PEN NO.	NO. FERTILE EGGS	INCREASE IN MORTALITY			
				1–6	7–13	14–17	18–22			1–6	7–13	14–17	18–22
1935	2/19–4/ 9	13	368	4.08	1.98	2.89	10.12	16	555	−0.48	2.32	1.02	2.28
	4/16–6/ 4	13	434	2.30	0.94	0.71	3.36	16	625	0.74	0.55	−0.04	2.88
	2/19–4/ 9	14	387	2.07	4.75	4.99	12.83	15	269	3.13	−3.97	−3.01	1.69
	4/16–6/ 4	14	402	2.74	1.79	1.30	9.23	15	194	2.41	−0.16	0.36	0.88
1936	4/21–7/14	12	665	3.61	1.87	0.95	7.06	13	700	1.96	1.46	0.15	6.55
	3/ 3–4/12	14	343	2.04	2.38	2.74	12.23	15	250	0.76	0.09	−2.32	1.75
				Mean increase (each with standard error of 0.54)						1.42	0.05	−0.64	2.67

mortality for eggs from normal birds is given in the left half of the table and the increased mortality of the eggs from their Creeper sisters in the right half. These differences in the right half of the table have been averaged without weighting for unequal numbers of eggs. The eggs from the Creeper birds showed a small but consistently higher mortality in the last stages of development, from the eighteenth to the twenty-second day, but during the first 17 days frequently had a lower mortality than the eggs from their normal sisters. The mean difference in the last stage was clearly significant ($t=4.95$, $P<.01$)

but not for the first three periods. Since the Creeper factor had no striking effect upon the mortality from the first to the end of the seventeenth day of development, these stages have not been considered further. Of the total mortality in the embryonic period, 55 percent occurred from the eighteenth day through hatching, and during this phase the eggs laid by Creeper mothers showed the higher mortality.

DIFFERENCES BETWEEN SIBSHIPS

Though differing in the rate of survival, the eggs on each side of table 2 were equally heterozygous for the Creeper factor. In order to balance the rest of the chromosomal complex more fully, it is desirable to shift from the egg as the unit to the individual sibship of hens laying the eggs. The sibships, however, were not represented equally in the paired pens, and percentage mortalities based upon the relatively small number of eggs for each hen within a test period—from one to 63—had comparatively large sampling errors. Since the percentage of chicks hatched was frequently erratic where the hen laid very few eggs, further calculations were restricted to individuals which during a test period laid nine or more eggs which survived the seventeenth day of incubation.

It was next determined whether or not the mortality of the eggs laid by sister birds of the same genotype differed more than could be attributed to chance. If these varied only within the limits expected from the binomial distribution, the records of dead and hatched eggs for each sibship, pen, and period could be totalled without regard to the number of hens laying the eggs. The homogeneity among the hens forming all such groups has been tested by χ^2, as shown in table 3. In groups containing only two birds, χ^2 was computed with

TABLE 3

Test for homogeneity in the late mortality of eggs from two to four hens of the same sibship, pen and laying period; restricted to birds laying nine or more eggs which completed 17 days of development.

YEAR	CREEPER MALE × NORMAL FEMALES					NORMAL MALE × CREEPER FEMALES				
	PEN NO.	NO. OF GROUPS	χ^2	DEGREES FREEDOM	P	PEN NO.	NO. OF GROUPS	χ^2	DEGREES FREEDOM	P
1935	13	9	15.98*	15	.39	16	11	28.32	26	.34
	14	9	16.68	14	.27	15	5	7.80	9	.55
1936	12	6	20.32*	10	.027	13	6	27.71*	15	.023
	14	6	16.14*	9	.065	15	4	7.29	5	.20

* Contains one heterogeneous group of three or four hens, where the omission of a single bird reduced the variability of the group and of the pen to within the range expected for homogeneity.

Yates' correction for continuity (FISHER 1938). Summing all groups, the combined $\chi^2 = 140.24$ with 103 degrees of freedom, indicating a significant heterogeneity ($P = .016$). However, only four of the 56 component groups showed an

internal heterogeneity, and with the omission of the eggs from one hen in each of these four groups, the remaining two or three fowls formed a homogeneous set. With these four omissions, the combined χ^2 dropped to 108.47 with 99 degrees of freedom and $P = .49$.

Since so predominant a part of the variation within groups fell within the sampling error, all cases in which the hens laid nine or more eggs completing 17 days of development have been combined by sibships within pens and periods. These included the four apparently heterogeneous birds referred to in table 3. Of the 33 paired comparisons within groups which met this first requirement, nine, on one side of the comparison, had only a single bird of either Creeper or normal stock laying fewer than 25 eggs. As would be expected from their sampling errors, these nine comparisons were by no means consistent and not suitable for further analysis. Hence they have been omitted from table 4, which shows the embryo mortality in all sibship-periods where every hen laid at least nine eggs and both sides of the comparison totalled at least 25 eggs completing 17 days of development. In these 24 paired comparisons, the relation of embryo survival to the Creeper factor is as nearly free of unequal action from other genes as it can be made.

ANALYSIS OF MORTALITY DIFFERENCES

The analysis of the increase in mortality in table 4 posed two complications: (1) the level of mortality differed from one sibship or period to another and (2) the percentage mortalities were based upon numbers varying from 27 to 146 eggs. Hence constant differences in the two percentage mortalities would not be expected for equal effects of the Creeper factor, and some differences would have a higher validity than others. In the range covered by these experiments percentages do not form an equal-interval scale, in that the standard deviation of the binomial distribution decreases markedly as the expected percentage approaches zero. This can be corrected by transforming percentages to angles for which the variance is independent of the expected value and determined only by the number of observations (BLISS 1938). Hence the percentage egg mortalities have been changed to angles by a table of transformation for the normal hens and their Creeper sisters in each row of table 4 and the former subtracted from the latter to obtain the differences in terms of angles. To avoid the discontinuity at 0 percent in three cases, the expedient suggested by BARTLETT (1937) has been adopted of substituting $\frac{1}{4}$ for 0 in computing the mortality to be changed to angles. Each difference then had a weight proportional to $W = [N_1N_2/(N_1+N_2)]$ (the last column of table 4) where N_1 is the number of eggs from normal females and N_2 that from their Creeper sisters. These weights varied from 14 to 73, despite the omission of groups with few eggs.

An analysis of variance in terms of the Creeper effect between and within pens showed no difference in the mean effect which could be attributed to pens. Hence all sibship-period groups have been combined into a single weighted mean difference of $5.240 \pm 1.236°$, from which $t = 4.24$ with 23 degrees of free-

dom and $P < .001$. Hence there can be no doubt that, as far as embryo survival is concerned, the eggs laid by Creeper hens are at a disadvantage in comparison with the eggs laid by their normal sibs.

TABLE 4

Mortality by sibships; number of eggs is the total surviving the seventeenth day of incubation, omitting sibships with fewer than 2.5 eggs from either type of female.

YEAR AND PENS	PERIOD	SIB-SHIP NO.	NORMAL FEMALES			CREEPER SISTERS			INCREASE IN MORTALITY		
			HENS NUMBER	EGGS NUMBER	DEAD PERCENTAGE	HENS NUMBER	EGGS NUMBER	DEAD PERCENTAGE	FROM PERCENTAGE	FROM ANGLES	WEIGHT
1935	2/19–4/ 9	2444	2	42	0	3	58	10.34	10.34	14.34	24.4
13–16		2445	3	67	10.45	4	113	14.16	3.71	3.25	42.1
		2446	3	61	11.48	4	102	19.61	8.13	6.49	38.2
		2447	4	98	6.12*	4	112	11.61	5.49	5.60	52.3
	4/16–6/ 4	2444	1	37	0	3	93	5.38	5.38	8.70	26.5
		2445	3	84	1.19	4	134	8.21	7.02	10.39	51.6
		2446	3	100	6.00	4	111	7.21	1.21	1.39	52.6
		2447	2	63	4.76	4	106	8.49	3.73	4.34	39.5
		2454	1	30	3.33	1	27	3.70	0.37	0.57	14.2
1935	2/19–4/ 9	2421	2	44	6.82	2	43	2.33	−4.49	−6.36	21.7
14–15		2424	4	82	8.54	4	75	25.33	16.79	13.21	39.2
		2428	2	54	20.37	3	47	17.02	−3.35	−2.46	25.1
	4/16–6/ 4	2421	1	28	0	2	47	4.26	4.26	6.49	17.5
		2424	3	69	11.59	3	76	13.16	1.57	1.37	36.2
1936	4/21–7/14	2619	3	104	13.46	2	75	6.67	−6.79	−6.55	43.6
12–13		2621	3	146	2.05*	4	145	12.41	10.36	12.40	72.7
		2622	2	30	10.00	4	111	18.02	8.02	6.68	23.6
		2627	3	123	10.57	4	114	14.91*	4.34	3.75	50.2
		2638	3	129	5.43	3	82	7.32	1.89	2.22	50.1
		2642	2	77	3.90	4	99	17.17	13.27	13.09	43.3
1936	3/ 3–4/14	2952	3	62	17.74	3	60	11.67	−6.07	−4.94	30.5
14–15		2953	2	49	8.16	2	46	23.91	15.75	12.68	23.7
		2954	2	58	8.62	2	32	9.38	0.76	0.76	20.6
		2955	3	56	5.36	2	52	13.46	8.10	8.14	27.0

* Heterogeneous group, all others homogeneous by χ^2 test.

The magnitude of the angular difference in embryo mortality may be understood more readily if translated back into percentages. This cannot be done directly, however, since the percentage corresponding to a difference of one degree depends upon its location on the percentage scale. The simplest procedure, perhaps, is to compute the weighted mean mortality for normal birds in degrees (14.98°), add the weighted mean difference to obtain the "expected" value for Creeper birds ($14.98 + 5.24 = 20.22°$), convert both to percentages (6.68 and 11.94 percent), and from their difference find 5.26 percent as the value corresponding to the mean angular difference of 5.24°. Their close agreement is fortuitous, for if we had chosen the mean mortality of eggs from the normal pen 13 during the second period of 1935—3.18 percent—as our standard, the expected increase due to the use of Creeper hens would be 3.98 per-

cent. Alternatively, with the normal pen 14 in 1936 as the standard, the mean mortality of 9.77 percent would be increased by an expected 6.07 percent in the Creeper siblings. On the equalized angular scale, on the other hand, the increased mortality of eggs from the Creeper fowls presumably is independent of the normal mortality.

If the significant increase in late embryo mortality, which occurs in eggs laid by Creeper hens, were due to the presence of the Creeper factor previous to fertilization, then one should expect an excess of mortality of Creeper offspring in progenies of Creeper mothers as compared with Creeper offspring of their normal sisters. The relevant data are given in table 5. There is no con-

TABLE 5

Embryo mortality between 18–22 days of incubation, together with the number and percentage of Creepers among the dead embryos, for paired pens of reciprocal matings between Creeper and normal fowl.

FROM NORMAL MOTHERS					FROM CREEPER MOTHERS				
YEAR	PEN	TOTAL EMBRYOS	CREEPER EMBRYOS	CREEPER PERCENTAGE	YEAR	PEN	TOTAL EMBRYOS	CREEPER EMBRYOS	CREEPER PERCENTAGE
1935	13	48	25	52.1	1935	15	49	29	59.2
	14	86	51	59.3		16	96	47	49.0
1936	12	56	38	67.9	1936	13	93	56	60.2
	14	39	23	59.0		15	34	21	61.8
Total		229	137	59.8			272	153	56.3

sistent difference between the reciprocal matings in respect to the percentage mortality of Creeper and normal embryos; in both types of matings Creeper embryos appear to be at a similar disadvantage. This conclusion is amply confirmed by a comparison of data from other reciprocal crosses. It must be concluded then that the increased embryonic mortality among eggs which have been laid by Creeper females has nothing to do with the genetic constitution of the eggs before fertilization.

COMPARISON OF SHELLS FROM CREEPER AND NORMAL

The only remaining alternative is an influence by the laying Creeper females on formation of their eggs. We do not have definite information at present with regard to the nature of this influence, but it appears likely that it is concerned with the calcium metabolism of Creeper hens. It is a well established fact that the skeletal calcium of laying hens serves as a reserve depot for shell formation (COMMON 1933, 1938, 1941; TYLER 1940; DEOBALD, LEASE, HART, and HALPIN 1936; MORGAN, MITCHELL, RINGROSE, and LEASE 1942), and it has been demonstrated that the endosteal bone trabeculae of the long bones undergo a cyclic destruction and rebuilding in association with egg lay-

ing and shell formation (BLOOM, BLOOM, DOMM, and MCLEAN 1940). The Creeper mutation has a specific effect on endosteal bone, greatly reducing the number of trabeculae in the long bones (LANDAUER 1931). It seems reasonable to assume that this deficiency results in less ample reserves of readily available skeletal calcium, and that this in turn may at times lead to irregular shell formation and death of the embryo.

In order to obtain some information on whether or not differences in shell formation exist between Creeper hens and their normal sisters, determinations of shell weight relative to weight of egg contents were made for eggs from the same matings which in 1935 were used for observations on viability. The means and coefficients of variability for these data are given in table 6. The

TABLE 6

Means of relative shell weight and their coefficients of variability for paired pens of normal and Creeper females.

MATING	N	WEIGHT OF SHELL IN PERCENTAGE OF WEIGHT OF EGG CONTENTS		
		MEAN	COEFFICIENT OF VARIABILITY	DIFFERENCE BETWEEN COEFFICIENTS OF VARIABILITY
Pen 13 Normal females	290	10.14±0.04	6.73±0.28	2.59±0.43
Pen 16 Creeper females	413	9.92±0.05	9.32±0.32	
Pen 14 Normal females	194	10.13±0.05	7.26±0.37	3.32±0.79
Pen 15 Creeper females	116	10.23±0.10	10.58±0.69	

egg and shell weights were obtained from eggs which had been laid either before or after the period during which eggs were incubated for the determination of embryo mortality and of the percentage of chicks which hatched. The means and coefficients of variability were calculated from all data for each pen, irrespective of the number of eggs contributed by individual females. Inasmuch as percentage shell weight appears to be reasonably uniform when the animals are on an adequate diet, it is believed that the constants thus calculated give a sufficiently reliable indication of the true situation.

Two conclusions may be drawn from the data in table 6: (1) the means of relative shell weight do not differ significantly between eggs laid by Creeper females and by their normal sisters; (2) the variability of percentage shell weight is definitely greater for the eggs of Creeper females than for those of normal females. Since there is no difference in mean percentage shell weight, it

must be concluded that eggs laid by Creeper females have at times relatively heavier, at other times relatively lighter shells than is typical for normal females.

In view of our knowledge concerning the defectiveness of endosteal bone in Creeper fowl and of the established importance of this trabecular bone as a calcium depot for shell formation, one should expect just such a situation. Due to the scarcity of trabecular bone in Creepers, excess amounts of dietary calcium presumably cannot so readily be stored for future use as in normal fowl, but instead may be applied directly and in larger than usual amounts to formation of the egg shell. At other times, when food calcium is at a low level, the defective endosteum cannot supply sufficient amounts of calcium, resulting in lighter shells than normal. It is known that a relation exists between thickness of the shell and the rate at which water evaporates from eggs during incubation (MURRAY 1925), and it is also known that thickness of the shell has an influence on embryo survival and hatching (WILHELM 1939). Tentatively we may conclude that the excessive variations in shell deposition account for the increased embryo mortality in eggs laid by Creeper females, but our data do not show whether the abnormally heavy shells alone, or the abnormally light shells, or both are responsible for this indirect effect of the Creeper mutation on embryonic mortality.

SUMMARY

The embryo mortality has been compared in progenies from reciprocal crosses of Creeper and normal male siblings mated to normal and Creeper female siblings, respectively. Although of equivalent chromosomal constitution, the mortality in eggs laid by the Creeper birds averaged about 5.3 percent greater than in the eggs of their normal sisters, a significant effect which has been localized in the last five days of the incubation period.

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EFFECTS OF GENOME BALANCE, POLYPLOIDY, AND SINGLE EXTRA CHROMOSOMES ON SIZE IN NICOTIANA

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INTRODUCTION

SINCE doubling or otherwise altering the number of chromosomes in many species of plants can be accomplished by relatively simple laboratory techniques, it is possible to utilize these methods to provide new material for studying the inheritance of "quantitative characters." In types especially favorable for analyses of this sort the influence of extra single chromosomes or chromosomal complements on size, shape, and other measurable characteristics under polygenic control can now be investigated on a wider scale. Genetic analysis is, in a sense, simplified by the use of this method, since a discontinuous rather than the usual continuous segregation is obtained.

Some significant contributions along these lines have already been made with plant material. The relation between different proportions of specific genomes and the expression of quantitative characters has been investigated in polyploid hybrids between Raphanus and Brassica (KARPECHENKO 1927), Physcomitrium and Funaria (WETTSTEIN 1928), *Tradescantia subaspera* and *T. canaliculata* (ANDERSON 1936), Zea and Tripsacum (MANGELSDORF and REEVES 1939). Similar studies have been made on types with single extra chromosomes in *Datura stramonium* (SINNOTT, HOUGHTALING, and BLAKESLEE 1934) and *Nicotiana sylvestris* (GOODSPEED and AVERY 1939). Morphological changes due to the addition of chromosomes of one species onto the background of another have been described: notably, of Tripsacum chromosomes on *Zea mays* (MANGELSDORF and REEVES 1939) and of *Secale cereale* chromosomes on *Triticum vulgare* (O'MARA 1940).

The present investigations were undertaken in order to establish the precise relation between genome balance and corolla size in certain interspecific polyploid hybrids of Nicotiana and to study quantitatively the effects of extra single chromosomes from *N. Langsdorffii*. The significance of the studies depends on the extent to which they may bear on the general problem of the inheritance of quantitative characters.

MATERIALS AND METHODS

The strains of *Nicotiana Langsdorffii* Weinm. (n=9) and *N. Sanderae* Hort. ex. W. Wats. (n=9) used in these experiments were inbred for many generations and are highly homozygous. They have been described and illustrated in previous publications (SMITH 1937, 1943).

Doubled chromosome forms of each species and of the F_1 *Langsdorffii* $\times$*Sanderae* were produced by treating pregerminated seeds with 0.2 or 0.4

percent aqueous solution of colchicine for from six to 24 hours. The other chromosomal types listed in table 1 and illustrated in plate I were obtained by making appropriate crosses among the original diploid and double chromosome forms. The symbol L is used throughout this paper to signify a haploid complement of *N. Langsdorffii* and S a complement of *N. Sanderae.*

The LLLS type was produced by crossing a tetraploid *Langsdorffii* (LLLL) with the amphidiploid (LLSS); the LLS type by crossing 4n *Langsdorffii* with 2n *Sanderae* (SS); LSS by crossing the amphidiploid with 2n *Sanderae;* and

TABLE 1

Corolla size in chromosomal types involving different proportions of genomes of Nicotiana Langsdorffii (L) and N. Sanderae (S).

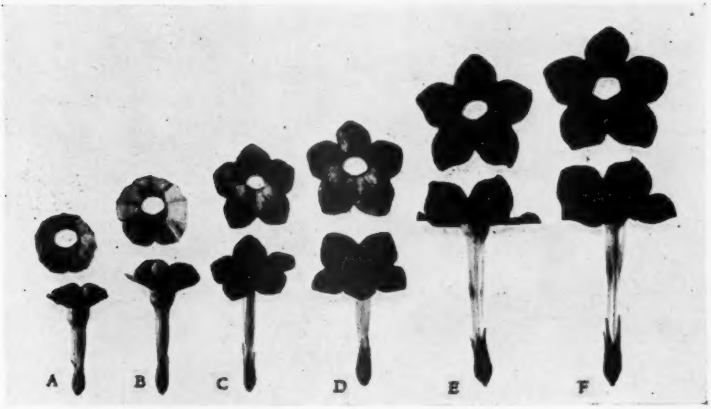
GENOTYPE	NO. INDIV.	CHROM. NO.	PERCENT *Sanderae*	COROLLA MEASUREMENTS					
				TUBE BASE		THROAT		LIMB	
				Mean mm	C.V. %	Mean mm	C.V. %	Mean mm	C.V. %
LL	113	18	0.0	4.3	9.3	14.8	3.5	5.6	8.9
LLLL	66	36	0.0	5.0	7.4	15.3	3.6	5.2	7.1
LL×LS	228	18	±25.0	8.4	28.0	16.9	7.6	9.0	17.7
LLLS	63	36	25.0	10.3	12.9	19.0	5.6	8.4	8.9
LLS	58	27	33.3	11.6	8.9	20.1	6.1	10.3	7.9
LS	174	18	50.0	16.5	6.9	19.7	4.2	13.6	8.6
LLSS	36	36	50.0	17.5	9.6	21.8	6.1	13.3	8.9
LSS	21	27	66.6	29.8	6.6	23.4	5.8	18.6	8.3
LS×SS	206	18	±75.0	30.5	21.4	18.2	10.7	20.8	13.2
LSSS	3	36	75.0	36.0	2.8	23.2	7.5	22.3	6.9
SS	89	18	100.0	49.7	8.3	20.4	7.9	28.8	9.4
SSSS	6	36	100.0	58.3	4.0	24.7	5.6	30.5	4.5
LS+Langs. chrom. I	50	19	47.4	13.4	5.6	20.6	4.8	11.6	6.2
LS+Langs. chrom. II	12	19	47.4	15.6	5.1	16.4	5.7	10.1	6.6
LS+Langs. chrom. III	35	19	47.4	16.9	3.9	21.7	3.4	12.7	5.0
LS+Langs. chrom. V	6	19	47.4	12.2	9.6	18.2	6.4	11.7	7.0
LS+Langs. chrom. VII	26	19	47.4	14.0	6.5	19.2	3.4	11.0	8.1

LSSS by crossing the amphidiploid with 4n *Sanderae* (SSSS). Some off-type plants were always found among progeny of polyploid parentage, but they were easily recognized and discarded.

Data on backcrosses of the F_1 to *Langsdorffii* (LL×LS) and to *Sanderae* (LS×SS) were obtained for comparison with measurements on certain polyploid forms.

Types with single extra *Langsdorffii* chromosomes on the background of the F_1 were produced by crossing different 2n+1 *Langsdorffii* plants with 2n *Sanderae* and were also found among offspring of the cross 3n *Langsdorffii* by 2n *Sanderae.* The F_1 was used as a background rather than *N. Langsdorffii,* itself, since the flowers are larger and any changes in size more obvious. The

same general effects can be observed, however, in the 2n+1 plants. The extra chromosomes have not yet been identified morphologically with those described by AVERY (1938) nor distinguished from each other, but the phenotypes are distinct and have been reproduced a number of times. The writer is working to obtain all primary trisomics of *N. Langsdorffii* and to investigate their effect on quantitative characters. Therefore, the results reported on the five 19-chromosome types listed in tables 1 and 3 are preliminary.



EXPLANATION OF PLATE I

Corollas of hybrids involving different proportions of genomes from *N. Langsdorffii* (L) and *N. Sanderae* (S). ×¼. Figure A.—LLLS. Figure B.—LLS. Figure C.—LS. Figure D.—LLSS. Figure E.—LSS. Figure F.—LSSS.

The characteristic studied in these experiments was corolla size since the parental species differ conspicuously in this respect. Measurements were made on the following regions of the corolla that together comprise the overall flower length (figure 1). The tube base was measured from the basal end of the flower to the insertion point of the anthers; the throat from the insertion point to the region where the limb flares out abruptly; and the limb from this point to the tip of the longest lobe. These regions are morphologically, cytologically, and physiologically distinct (NAGEL 1939). The tube base and limb are much longer in *N. Sanderae* than in *N. Langsdorffii*, but the difference in throat length between the two species is relatively small.

RESULTS AND DISCUSSION

Relation between genome balance and size

Average corolla measurements on the types studied are arranged in table 1, and flowers of the different hybrids are shown in plate I. The latter contain 25, 33, 50, 67, and 75 percent *Sanderae* germplasm. It is at once apparent from the data and illustrations that there was a progressive increase in corolla size

with each proportionate increase in the amount of *Sanderae* present. This general correlation was evident for all regions of the corolla.

In order to investigate the relationship further, graphs were constructed as shown in figure 2, by spacing points along the abscissa according to the relative amounts of *N. Sanderae* germplasm in the type represented; for example, average measurements on LLLS were placed one quarter the distance from 4n *Langsdorffii* to 4n *Sanderae*. The ordinate was arranged on a logarithmic scale, base 10, since this spacing gave approximately straight lines, whereas on an arithmetic scale the relationships were curvilinear.

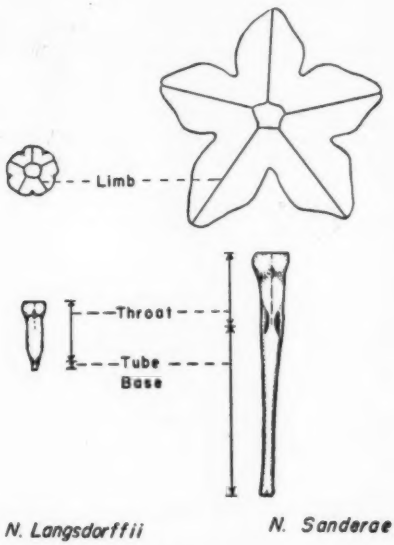


FIGURE 1.—Corollas of *N. Langsdorffii* and *N. Sanderae* showing the three measurements of size used in these experiments. ×½.

Effects due to variation in total number of chromosomes were kept to a minimum by using the tetraploid values for the species and the amphidiploid value instead of that of the F_1.

It is evident from figure 2 that the length of the tube base and limb of a corolla was directly proportional, on a logarithmic scale, to the relative amounts of *Langsdorffii* and *Sanderae* genomes present in each polyploid genotype. It follows from this observation that each specific complement has a constant multiplying effect on corolla size, regardless of the other genomes present; and in a polyploid hybrid, the balance between the number of genome effects from each species determines the size of the corolla. This relation was not obvious for the throat region where there is a comparatively small difference between the parental species.

The fact that the size components contributed by each genome accumulated their effects geometrically substantiates earlier evidence, based on analyses of diploid hybrids, that the size genes in this material have geometric effects (EAST 1913, SMITH 1937, CHARLES and SMITH 1939). In accordance with these results corolla size in a polyploid hybrid should be equal to the geometric mean of the genome values. If we assign the value of 5.0 for the tube base of L and 58.3 for S (table 1), the calculated mean for LLS would be $\sqrt[3]{5.0\times5.0\times58.3}$ or 11.3. The observed mean was 11.6 mm. Similarly, the calculated mean for LLLS would be $\sqrt[4]{5.0\times5.0\times5.0\times58.3}$ or 9.2, and the observed mean was

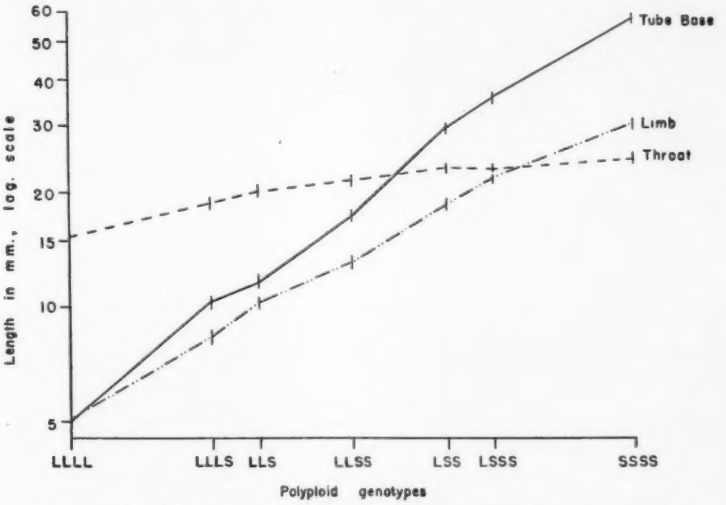


FIGURE 2.—Relation between relative proportions of genomes from *N. Langsdorffii* (L) and *N. Sanderae* (S) and the size of three different regions of the corolla.

10.3 mm. Agreement between the theoretical and experimental values is fairly close and is therefore further substantiation of the hypothesis that the size genes act in a geometric manner and that the numerical value of each specific genome remains constant in the different polyploid hybrids.

The relation between genome balance and corolla size was demonstrated in another way by adding complements of each species, one at a time, to the background of the F_1, as shown for the tube base in table 2. Each addition of a *Sanderae* complement caused a progressive increase in the length of the tube base and each addition of *Langsdorffii* a decrease. Furthermore, the change and rate of change resulting from the addition of the second complement in each series was less than that from the first. The experimental results are similar to the calculated rates of change in percentage of *N. Sanderae* germplasm (table 2) and are compatible with the conclusion that corolla size is determined by the relative proportions of the specific chromosomal complements.

Theoretical genotypic values for the hybrids listed in table 2 were calculated on the basis of a constant average increment, and their rates of change were compared with those obtained experimentally. It was found that the empirical rate was greater than expected theoretically when a *Sanderae* complement was added to the F_1 and was smaller when *Langsdorffii* complements were added. Since the chromosome number was increased from 18 to 27 to 36 in each series in table 2, and polyploidy is known to make the tube base longer (see below), then the size of the triploid and tetraploid hybrids was made larger independently of the influence of genome balance. Thus polyploidy augmented the size-increasing effect of additional *Sanderae* complements and diminished the size-decreasing effect of additional *Langsdorffii* complements; so that in the former series the rate of change was greater than expected on the basis of genome balance and in the latter it was smaller.

TABLE 2

Effect on size of the corolla tube base of adding one and two chromosomal complements of N. Langsdorffii (L) or N. Sanderae (S) to the F_1.

GENOTYPE	MEAN IN MM	CHANGE	RATE OF CHANGE	PERCENTAGE *Sanderae*	CHANGE IN %	RATE OF CHANGE IN %
LS	16.5			50		
LSS	29.8	+13.3	0.81	67	+17	0.34
LSSS	36.0	+ 6.2	0.21	75	+ 8	0.12
LS	16.5			50		
LLS	11.6	− 4.9	0.30	33	−17	0.34
LLLS	10.3	− 1.3	0.11	25	− 8	0.24

The results shown in figure 2 and table 2 indicate that the differences in corolla size between the two species, in so far as they are genic, are controlled by genes with incomplete dominance (ANDERSON 1936). This conclusion is based on the argument that if dominant genes were contributed by *Sanderae* (*AA*), then LLLS (*aaaA*) would resemble LLS (*aaA*) or, if dominants were contributed by *Langsdorffii* (*BB*), LSS (*Bbb*) would resemble LSSS (*Bbbb*). Since these conditions were not realized, it was concluded that phenotypic incomplete dominance was dependent on the operation of "non-dominant" genes at each locus involved rather than on the counteracting effect of different dominant genes from each parent. It has been reported that size genes linked with certain genes for color in this material were "non-dominant in the sense that *Aa* has less effect than *AA*" (SMITH 1937). All these observations and conclusions are compatible with the genetic explanation that corolla size is determined by genes with geometric action; so that the contribution of each locus is equal to the geometric mean of the alleles, and ultimate size is determined by the product of these means.

Relation between chromosome number and size

Data were available for observing the effect of a doubled number of chromosomes on corolla size in *N. Langsdorffii*, *N. Sanderae*, and in different intermediate forms (table 3). Five pairs of comparisons were made between types

TABLE 3

Changes in size of Nicotiana corollas produced by a double number of chromosomes and by single extra chromosomes from N. Langsdorffii.

GENOTYPES COMPARED	TOTAL DEGREES OF FREEDOM	F REQUIRED FOR 99:1 ODDS	TUBE BASE		THROAT		LIMB	
			MEAN DIFF. IN MM	F OBTAINED	MEAN DIFF. IN MM	F OBTAINED	MEAN DIFF. IN MM	F OBTAINED
18 chrom. vs. 36 chrom. types								
LL vs. LLLL	178	6.76	+0.7	130.00	+0.5	30.96	−0.4	31.43
LL×LS vs. LLLS	290	6.72	+1.9	40.65	+2.1	145.65	−0.6	8.18
LS vs. LLSS	209	6.76	+1.0	19.83	+2.1	164.61	−0.3	1.41
LS×SS vs. LSSS	208	6.76	+5.5	2.09	+5.0	18.29	+1.5	0.89
SS vs. SSSS	94	6.90	+8.6	25.33	+4.3	30.16	+1.7	2.45
F_1 vs. F_1+1 extra Langs. chrom.								
LS vs. LS+Langs. I	223	6.76	−3.1	303.25	+0.9	55.46	−2.0	123.20
LS vs. LS+Langs. III	208	6.76	+0.4	4.61	+1.0	195.43	−0.9	19.65
LS vs. LS+Langs. V	179	6.76	−4.3	85.41	−1.5	17.58	−1.9	15.64
LS vs. LS+Langs. VII	199	6.76	−2.5	112.48	−0.5	5.67	−2.6	117.05
LS vs. LS+Langs. II	185	6.76	−0.9	7.52	−3.3	170.49	−3.5	104.45

with 18 and with 36 chromosomes that had the same relative proportion of genomes—namely, LL vs. LLLL, LL×LS vs. LLLS, LS vs. LLSS, LS×SS vs. LSSS, and SS. vs. SSSS. In each pair the tube base and throat were longer in the types with 36 chromosomes than in those with 18. The increase was significant according to the F value required for 99:1 odds in each comparison with the exception of the tube base in LS×SS vs. LSSS where a smaller F was obtained owing to the large variance within the segregating backcross population. Larger size of the tube base and throat was, no doubt, a result of the increase in cell size caused by polyploidy. The effect of chromosome doubling on the tube base was more nearly multiplicative than additive and the multiplying value was approximately 1.17. For example, 4.3 (LL)×1.17 is equal to 5.0 which is the experimental mean for LLLL; and 49.7 (SS)×1.17 is 58.1 compared to 58.3, the observed mean for SSSS. This multiplier is slightly less than 1.26, the cube root of 2, which is the linear increase in solids whose volume is doubled. The probable explanation for the smaller value obtained is that although the cells of the tube base were doubled in volume, their length was increased proportionately less than their width.

The increase in length of the throat region due to a double number of chromosomes was greater in some cases than that due to a higher percentage of *Sanderae* germplasm. For example, the average throat measurement for LLSS (50 percent *Sanderae*, 36 chromosomes) was 21.8 mm and for SS (100 percent *Sanderae*, 18 chromosomes) was 20.4 mm. This difference was significant, the F value being greater than required for 999:1 odds.

There was no significant difference in the limb measurement between 18- and 36-chromosome types that had 50, 75 and 100 percent *Sanderae* germplasm, but in those with 0 and 25 percent the double-chromosome forms had a significantly shorter limb. The only explanation offered for this result is that the length of the limb is unaffected or adversely affected by larger cell size. However, other features that were not measured in these experiments are noticeably affected. For example, the sinuses between the lobes are more filled with tissue (especially in 4n *Sanderae*) and the overall spread of the limb is greater, owing to the fact that the tubular part of the flower is wider in polyploids (SMITH 1943).

Effect of single extra chromosomes

The effects on corolla size of five different single extra chromosomes from *N. Langsdorffii* on the background of the F_1 *Langsdorffii*×*Sanderae* were studied. Average measurements on the three selected regions of the flower for each type are shown in table 1. Comparisons between these means and the corresponding ones of the F_1 are shown in table 3. Any significant difference in size was considered to be due to the effect of genes located in the extra chromosome involved.

Three of the chromosomes, designated as II, V and VII, caused a decrease in the size of all the measured regions of the corolla. The differences were significant according to the F value required for odds of 99:1 with one exception, concerning the effect of chromosome VII on the throat, which, however, was significant at the 19:1 level. The other two chromosomes investigated—namely, I and III—caused an increase in the size of one region of the corolla and a decrease in another. From these results the following conclusions were drawn, which should hold true so far as gene action can be judged by the effect of extra dosages.

Each of the chromosomes contains genes that influence corolla size.

The small-flowered parent, *N. Langsdorffii*, contains some genes for increasing size.

The genes do not necessarily determine size as a whole since two of the extra chromosomes each affected one part of the flower in a plus direction and another part in a minus direction. It is possible, however, that these genes affect the whole corolla but change the size of different parts in different ways. On the other hand, they may have only local effects.

There are genes for small size that have an active reducing effect, since corollas of the F_1 plus certain *Langsdorffii* chromosomes (or complements) are smaller than those of the F_1, itself. The same result can be observed on the background of *N. Langsdorffii*. Size, therefore, cannot be considered as de-

pendent only on an accumulation of genes that cause increases. A possible physiological interpretation of the operation of the reducing genes is suggested by the results of NAGEL (1939) who found evidence of inactivation of growth hormones in corollas of *N. Langsdorffii*. According to the hypothesis of geometric effects, these genes would be considered as having multiplying values of less than one.

For the purpose of illustrating the phenotypic results of geometric gene action let us assume that a large parent, PP, and a small one, P′P′, have a common residual genotypic value of K = 10 and differ at two loci for genes with the multiplying values of a = 5.8 and b′ = 0.5. Their corresponding alleles, a′ and b, are assumed to have no influence on size. The effective weight of each locus will be equal to the geometric mean of the alleles involved. Then:

$PP = K.aa.bb = 10 \times \sqrt{5.8 \times 5.8} \times \sqrt{1 \times 1} = 58.0$

$P'P' = K.a'a'.b'b' = 10 \times \sqrt{1 \times 1} \times \sqrt{.5 \times .5} = 5.0$

$PP' = K.aa'.bb' = 10 \times \sqrt{5.8 \times 1} \times \sqrt{1 \times .5} = 17.0$ (or $\sqrt{58 \times 5}$)

$PP'P' = K.aa'a'.bb'b' = 10 \times \sqrt[3]{5.8 \times 1 \times 1} \times \sqrt[3]{1 \times .5 \times .5} = 11.3$ (or $\sqrt[3]{58 \times 5 \times 5}$)

$PP'+b' = K.aa'.bb'b' = 10 \times \sqrt{5.8 \times 1} \times \sqrt[3]{1 \times .5 \times .5} = 15.2$

The above hypothetical scheme can be considered as a very rough and simplified picture of the genetic basis for the results on tube base in table 1. PP′P′ corresponds to the triploid hybrid LLS and PP′+b′ to the F_1 plus *Langsdorffii* chromosome II, for example. It seems likely that the many genes determining size may differ in degree of dominance and type of interactions as well as in magnitude of effect; however, the results obtained so far with this material can be explained adequately on the basis of a simple geometric action of genes which differ only in their individual numerical value.

SUMMARY

Certain crosses were made among 2n and 4n forms of a small-flowered species, *Nicotiana Langsdorffii* (n = L), a large-flowered species, *N. Sanderae* (n = S), and their amphidiploid (LLSS) in order to produce the following intermediate polyploid hybrids: LLLS, LLS, LSS, and LSSS. The size of three different regions of the corolla was measured in each type. A direct relationship was found between genome balance and corolla size. Changes in the proportion of L to S have more nearly a multiplicative than an additive effect on the tube base and limb of the flower. The specific genome values apparently remained constant and were accumulated geometrically so that the size in a polyploid hybrid was equal to their geometric mean.

The influence of a double number of chromosomes on corolla size was observed by comparing the following 18- and corresponding 36-chromosome types which have the same relative proportion of specific germplasms: LL vs. LLLL, LL×LS vs. LLLS, LS vs. LLSS, LS×SS vs. LSSS, and SS vs. SSSS. The length of the tubular part of the corolla was increased by polyploidy but the limb measurement was affected adversely or remained unaffected. Polyploidy has more nearly a multiplicative than an additive effect on the length of the tube base.

The effects of five different single extra *Langsdorffii* chromosomes, phenotypically detectable, were studied on the background of the F_1 with *Sanderae*. Three of the extra chromosomes each caused a reduction in the size of all regions of the corolla. The other two caused an increase in one region and decrease in another. It was concluded that each of the chromosomes contained genes that influenced corolla size though their effects were somewhat different. *N. Langsdorffii* contained some genes for increasing size. There are genes for small size that are not passive but play an active role in size inheritance.

All the results are compatible with the conclusion that size is determined by genes with geometric action in the sense that the independent contribution of each locus involved is equal to the geometric mean of the alleles, and these values are multiplied together so that the size of the end-product is equal to their cumulative geometric effect.

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THE X-RAY INDUCED MUTATION RATE IN POLLEN IN RELATION TO DOSAGE AND THE NUCLEAR CYCLE

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THE haploid cycle of plants offers some distinct advantages to genetic analysis. Here inheritance is not complicated by dominance relationships, and segregations are much less involved than in disomic inheritance. The greatly simplified problem of rearing large populations and the opportunities in some instances of tetrad analysis are other of the many well known reasons why new inherited characters of haplonts are eagerly sought. The recent work of BEADLE and TATUM (1941) on Neurospora exemplifies the rapid advancement possible in the genetics of haplonts and the interesting uses to which such knowledge may be applied.

It has been demonstrated that X-rays applied to microspores of Tradescantia shortly after meiosis induce mutations which are expressed in abortion and changes of size in the subsequently developed pollen grains (RICK 1942). For further understanding of this problem the statistical techniques need elaboration. Also, the response of pollen during stages of the cycle other than the critical stage immediately following meiosis deserves attention. Another aspect to be considered here is the response of these measures of mutation rate to varied X-ray dose.

TECHNIQUE

The frequency of X-ray-induced chromosomal aberrations has been well established for the microspore cycle in Tradescantia (SAX and SWANSON 1941). The duration of various stages of this cycle is also fairly well known. Thus, for the sake of comparison and convenience a clone of diploid *Tradescantia paludosa* Anderson and Woodson was used throughout this experiment.

In order to permit observations over a considerable period, inflorescences were irradiated while intact on the plant. Inflorescences were held in clusters in order to minimize dosage variation. Differences in dosage represent differences in intensity obtained by varying the distance from target to inflorescences according to the inverse square law. The distances from target to inflorescences were 35, 50, 71, and 100 cm with corresponding intensities of 100, 50, 25, and 12.5 r per minute. The period of treatment was five minutes, thus the doses achieved were 500, 250, 125, and 62.5 r. This was found from previous trials to be the most satisfactory dosage range for the purposes of this experiment. Very large samples are required to detect effects of doses lower than 62.5 r, and doses greater than 500 r injure flowers considerably and interrupt the normal rate of flowering. The source of X-rays was a Coolidge tube with tungsten target operating at 10 ma at a peak potential of 160 kv on a line voltage of 115 v. The radiation was unfiltered.

[1] Most of the data presented were collected by the writer while at the Biology Laboratories of HARVARD UNIVERSITY.

Pollen was collected from the treated inflorescences and the non-radiated controls two days before treatment and at daily intervals for the ensuing two months. Daily collections were taken because a new crop of flowers opens each morning and wilts later in the day. Thus collections were timed to the day with considerable accuracy. The pollen from a single flower was considered a unit collection and one sample was taken per collection.

By examination of the stage of development of microspores in the series of buds in several inflorescences and comparing these results with the rate of flowering of similar material, it was possible to estimate the time required for the different stages of the nuclear cycle in microspore development. It was found for the April to June period of this experiment that an average of 7.8 buds per inflorescence contained microspores in various stages between meiosis and mature pollen in open flowers, and a figure of 4.2 corresponds to the period of meiosis to metaphase of the microspore mitosis. The rate of flowering was 0.65 flowers per inflorescence per day. Therefore, 7.8/0.65 or approximately 12 days elapse between some stage of the meiotic divisions and anthesis, and 4.2/0.65 or approximately 6.5 days elapse between meiosis and microspore division. In the X-ray-treated buds the rate of flowering was approximately the same, and except for chromosomal aberrations, no deviations were noted in the orderly course of microspore mitosis. Apparently for the doses used here the timing of the normal microspore cycle remains relatively unaffected by the radiation. Experimental results to be noted later seem to support this conclusion.

Immediately prior to examination of a collection the pollen was mounted in aceto-carmine on a slide and covered by a coverglass mounted on pieces of broken coverglass in order to prevent any distortion of the pollen. By using aceto-carmine it is possible in the same collection to determine pollen abortion and to measure lengths of normal grains without risk of measuring aborted grains. In this medium grains swell until completely expanded from their normally deflated condition. It is improbable that measured size differences might be due to differences in ability of the grains to swell under these conditions, as Müntzing (1928) has shown in comparisons of pollen samples from the same source mounted in glycerine and in water. The pollen grain of Tradescantia expands in aceto-carmine to an elongate reniform shape. Length of grain was found to be the most useful dimension and for the purposes of this study is considered an adequate index of pollen grain size.

In preliminary experiments it soon became apparent that in samples of 200 to 500 grains a significant difference could be demonstrated between treatments, but in many cases significant differences were also found between samples of the same treatment—far too many, in fact, to be accounted for solely by chance variation. Apparently the source of this variation is to be found within the flower, possibly within the anther. This variation is a measure of the extreme sensitivity of pollen grain formation to factors of the internal and external environment and has been recognized by numerous workers. It bears close resemblance to the variation encountered in studies of chromosomal aber-

rations of the microspore division following X-ray treatment (SAX 1940; FABERGÉ 1940).

In order to gain some idea of the nature of this internal variation, tests for homogeneity were applied to several typical groups of data. As an illustration the results of such tests applied to the data in table 2 are presented in table 1.

TABLE 1

Probabilities that all sample values within a treatment belong to the same theoretical population. (Tests for homogeneity applied to data summarized in table 2.)

LOT NO.	DAYS AFTER TREATMENT	TREATMENT	POLLEN ABORTION	LENGTH OF POLLEN GRAIN	
				MEAN	VARIANCE
9–25	7 to 10	500 r	0.1–0.5	>.05	<.01
9–26	7 to 10	250	0.3–0.5	>.05	0.5 –0.95
9–28	9 to 11	125	0.5–0.95	<.01	0.02–0.05
9–29	9 to 11	62.5	0.5–0.95	<.01	0.05–0.1
9–30	9 to 12	control	0.3–0.5	<.01	0.2 –0.5
9–25	time of rad.	500 r	<.01	<.01	—
9–26	time of rad.	250	0.5–0.95	<.01	>0.99

Here we are concerned with three statistics: percentage of pollen abortion, mean length of pollen grain, and variance of grain lengths. Tests were applied that would indicate the probability that all of the values obtained in a given treatment were drawn from the same infinite population. Thus for percentage pollen abortion, the χ^2 test was applied (SNEDECOR 1940). To mean lengths was applied the criterion, $z = n\, s_m^2/s_p^2$ with $(k-1)$ degrees of freedom for the numerator and $k(n-1)$ degrees of freedom for the denominator, where s_m^2 refers to the variance between sample means, s_p^2, to the mean variance of the samples, k, to the number of samples, and n, to the number of grains per sample. For testing homogeneity of variances, the test devised by BARTLETT (1937) was applied. A sample of 25 was adopted for calculation of mean length and 100 for pollen abortion. If larger samples were taken, even smaller probabilities would be obtained. Thus, in spite of the smallness of sample size, a noteworthy heterogeneity exists. This is greatest for mean lengths and least for pollen abortion; hence, mean lengths are less useful in analysis of the mutation effect. There is a slight tendency for the heterogeneity to increase with X-ray dose except in the case of mean lengths. This effect on mean lengths might be expected because with increasing dose s_m^2 increases proportionally much less than s_p^2; in fact, the former increases somewhat less than the variance of pollen abortion and of variance of lengths.

Although this factor of internal variability seems somewhat more acute in the X-ray-treated material, it is still a factor of importance in untreated material. In the study of inheritance of length of pollen grain in *Pisum sativum* L. reported by RICK (1942) no X-rays had been applied. If BARTLETT'S (1937) test is applied to each set of sample variances, χ^2 values exceed the one percent

level in two lots and the five percent level in one other lot in the total of ten lots.

In many cases it was necessary to collect pollen from a treatment over a period of three or four days. It must be admitted that daily fluctuations were occasionally considerable and that these do contribute to the total heterogeneity; yet, when the tests were applied to collections of a single day in several treatments, thus excluding the day-to-day variation, significant values for heterogeneity were also obtained.

In order to compare statistically the treatment and control means, it was obviously necessary to adopt a valid estimate of error. In the presence of this appreciable internal variation, comparisons were based on the inter-sample variance rather than the inter-grain variance. As mentioned previously, a sample size of 25 grains was arbitrarily selected for lengths and one of 100 for pollen abortion. Possibly the efficiency of the technique could be improved by adopting even smaller samples.

POST-MEIOTIC TREATMENTS

The daily mean values for pollen abortion and variance of length were plotted for the two highest doses, 250 r and 500 r. The curves obtained are reproduced in figures 1 and 2. Each point is determined by from three to 16 samples, the mean being about eight. The percentage scale is adjusted to bring into greatest relief the fluctuations encountered before nearly complete sterility appeared on the twelfth day after treatment. Previous to the twelfth day the level of abortion changed, but such changes are relatively much smaller than this rise to 100 percent abortion. SAX (1938) described this sudden leap to complete sterility and attributed it to radiation during meiosis. This fact is verified in the present experiment, for the 12-day interval between radiation and the onset of nearly complete sterility agrees with the estimate already mentioned of the time elapsing between meiosis and anthesis. Since the two meiotic divisions probably occur within a 24 hour period, it is impossible to state with certainty how late in meiosis this effect continues, but one might speculate that it ends with meiotic prophase, the stage which is known to be so very susceptible to environmental influences. Whenever this extreme sensitivity ends, it has a very prolonged effect, for the return to a nearly normal level of pollen abortion is very slow. Further aspects of this meiotic sterility will be discussed in the next section.

It is evident in figures 1 and 2 and in other series that in the few days of collection preceding meiotic sterility both pollen abortion and variation in length are at new levels, well above the control level of the same period or the level of the treated lots in the few days immediately preceding and following treatment. There seems to be a slight gradual upward trend in these values as meiotic sterility is approached, suggesting that the effect is greater in material treated just subsequent to meiosis. This trend is not always obvious and is subject to considerable fluctuation. There seems to be little order to the daily fluctuations; pollen abortion seems to vary independently of variance of lengths, and the values for 500 r vary independently of those for 250 r. The

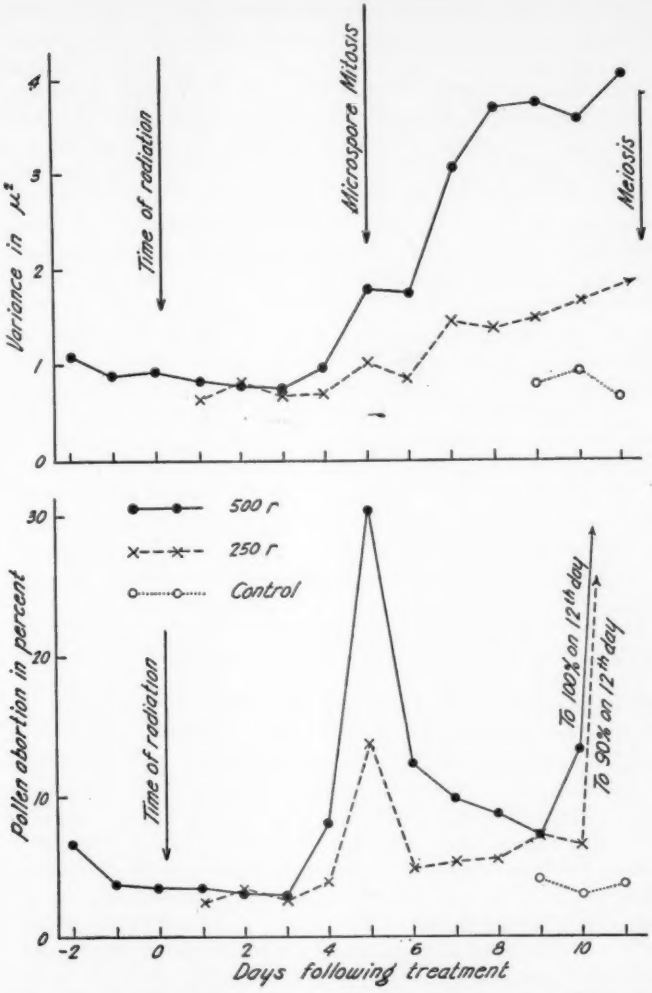

FIGURE 1 (above).—Daily mean values of variance of pollen grain length from the time of treatment to meiosis.

FIGURE 2 (below).—Daily mean values of pollen abortion.

fluctuations shown in figures 1 and 2 are somewhat greater than those usually obtained, and although the combining of values for the period of the seventh to eleventh day adds somewhat to the heterogeneity of the group, these data were massed in order to compare treatments.

Figure 2 reveals a sudden rise in pollen abortion centering on the fifth day

after treatment. It is unlikely that this very significant increase could be caused by some change in the environment other than X-rays since it appeared in both treatments and also in other series conducted in a different season; moreover, comparisons with the timing experiments in all cases show that this effect occurs at the time of microspore mitosis. This very close correspondence between sterility and nuclear divisions seems to substantiate the rate of development estimated in the timing experiments. It is also interesting to note that variation of lengths also rises on this same date, but the rise is of much smaller magnitude. The same relationship seems to exist during the period of high meiotic sterility. This differential reaction suggests that pollen abortion might be conditioned in a different manner during nuclear divisions than during the resting stage, although it could hardly be expected that variation of lengths could respond in proportion to the change in pollen abortion.

If these sudden rises in sterility represent a genetic effect of treatment, it follows that either chromosome breakage or intragenic change increases greatly in sensitivity to X-rays at some phase of microspore mitosis. If the response were of a strictly gene mutational nature, it should not occur, or at least it should be much less marked in diploid microspores. Actually, in diploid-tetraploid comparisons (RICK 1942), the diploid pollen of autotetraploid species suffers a rise in pollen abortion almost comparable to that of haploid pollen when treated at this period. During the period of meiotic sterility, also, diploid pollen is aborted to a great degree, though considerably less than haploid pollen. X-ray-induced gene mutations are predominantly recessive and hence would not be expressed in diploid pollen. In the light of these comparisons, therefore, an explanation of the sterility response on the basis of intragenic change seems unlikely.

It is more probable that the acute increase in sterility reflects a rise in chromosome sensitivity during prophase of microspore mitosis. A similar response in the frequency of chromosomal aberrations has been observed directly in this mitosis of Tradescantia by SAX and SWANSON (1941). They have compared the chromosome sensitivity during the nuclear cycle from meiosis to within eleven hours of metaphase and anaphase of the microspore mitosis and found the level of sensitivity highest approximately 20 hours before metaphase of the microspore mitosis; furthermore, the sensitivity at this stage is approximately three times as great as during the preceding resting stage. They did not extend their analysis any later than the eleventh hour, since thereafter the chromosomal response is of an entirely different nature—the "primary" effect of MARQUARDT (1938). This period has been explored, however, by MARSHAK (1937), who found a maximal chromosome sensitivity in somatic mitoses of root tips two to three hours before examination.

In such comparisons, however, it is invalid to assume that pollen abortion is induced entirely in the same manner at microspore mitosis as during the meiosis to mitosis resting stage, because, as demonstrated by previous work (RICK 1942) and by other evidence in this paper, the pollen abortion response does not resemble typical gene mutation when induced during the former period, but does so for the latter period. As mentioned later, the relation between

dosage and abortion for the period immediately following meiosis does not correspond to the squared type relation characteristic of chromosomal aberration (cf. SAX 1940 and others). Therefore, chromosomal aberrations probably play little part in determining this immediate post-meiotic abortion, whereas it is likely that they are involved to a great extent in the mitotic abortion. It is not to be inferred, however, that the abortion response at mitosis is a strictly aberrational response and abortion of the preceding period strictly intragenic. It may happen that the level of gene sensitivity remains approximately the same throughout, whereas the chromosomal sensitivity is at a very low level relative to gene sensitivity during the immediate post-meiotic period but during late prophase of microspore mitosis increases to a much higher level.

TABLE 2

The effects of X-ray dosage on the mutational response induced in pollen by treatment subsequent to meiosis.

LOT NO.	DAYS AFTER TREATMENT	TREATMENT	NUMBER OF SAMPLES	POLLEN ABORTION	LENGTH OF POLLEN GRAIN	
					MEAN	VARIANCE
MEASUREMENTS						
9–25	7 to 10	500 r	36	10.32±0.57%	46.11±0.11μ	3.803±0.186μ²
9–26	7 to 10	250	25	6.06±0.40	48.14±0.07	1.616±0.078
9–28	9 to 11	125	16	4.86±0.44	48.31±0.15	1.171±0.099
9–29	9 to 11	62.5	17	3.31±0.35	48.64±0.13	0.874±0.079
9–30	9 to 12	control	30	3.31±0.31	48.67±0.10	0.837±0.051
9–25	time of rad.†	500	53	3.32±0.29	48.84±0.07	0.945±0.034
9–26	time of rad.†	250	20	2.58±0.33	48.47±0.18	0.698±0.025
NET EFFECTS OF RADIATION BASED ON LOT #9–30 AS CONTROL						
9–25	7 to 10	500 r	36	7.01%*	−2.56μ*	2.966μ²*
9–26	7 to 10	250	25	2.75*	−0.53*	0.779*
9–28	9 to 11	125	16	1.55*	−0.36*	0.334*
9–29	9 to 11	62.5	17	0.00	−0.03	0.037

* Significant difference ($P \leq 0.05$) according to t test.

† Measurements taken at time of radiation may be considered as controls. See text for explanation.

A comparison of frequencies sheds little light on the aberration-abortion relationship. For any stage in the cycle the frequencies of gross chromosomal aberrations would be much more than sufficient to account for the levels of pollen abortion encountered. If chromosomal aberrations are directly concerned, it would follow that only certain ones are related to the abortion of microspores.

The data of post-meiotic treatments are summarized in table 2. Two sources of control data were taken: samples collected from untreated plants during the critical period—that is, immediately before the appearance of meiotic sterility —and samples from the plants treated with 250 and 500 r collected in a five-day period centering on the date of treatment. Judging from figures 1 and 2, the latter collections could be considered adequate controls, but in considera-

tion of daily fluctuations, it might be safer to rely on the former. The latter are necessary, however, as a check on the condition of inflorescences before treatment.

In this series as well as in others not reported here there is a consistent and statistically significant increase in pollen abortion and variance of length and decrease in length of grain as dose is increased. The mean values for pollen abortion and variance are plotted against X-ray dose in figure 3. The relation

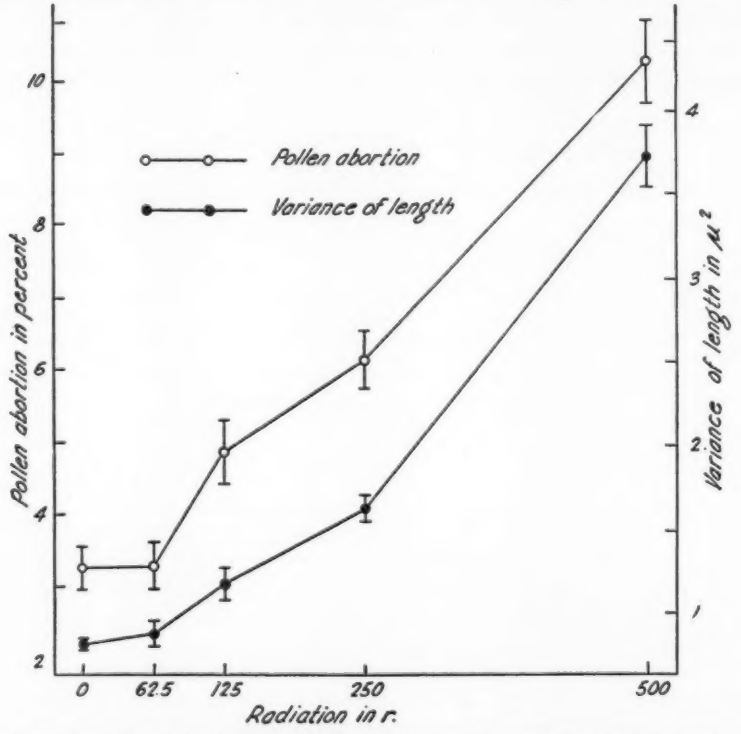

FIGURE 3.—The relationship between pollen abortion and X-ray dose and between variance of pollen grain length and X-ray dose in post-meiotic treatments. Vertical lines represent standard errors of mean values obtained.

between dose and pollen abortion is approximately linear, at least it does not deviate significantly from linearity. It does deviate markedly from the squared or near-squared relationship found for chromosomal aberrations induced during the same period (SAX 1940 and others). In this respect the response in pollen abortion resembles mutation (cf. TIMOFÉEFF-RESSOVSKY 1937) and not chromosomal aberration. Additional evidence is to be found in the fact that pollen abortion increases significantly in haploid but not in diploid microspores (RICK 1942).

The dosage relationship of variance of length lies between a linear and a squared relationship. Until more is known about the number, size of effect, and interaction of mutations concerned here, it is useless to attempt drawing any conclusions about this relationship.

The distributions of pollen grain lengths of controls, 250 r, and 500 r treatments were plotted in order to show other aspects of the mutational response. Without some correction for the great variation in mean lengths, such comparisons would be quite obscured. Accordingly, each sample distribution was adjusted until its mean coincided with the treatment mean whereafter the adjusted distributions were added. These three curves are shown in figure 4. Each curve is based on approximately 1000 grains. It is strikingly evident that

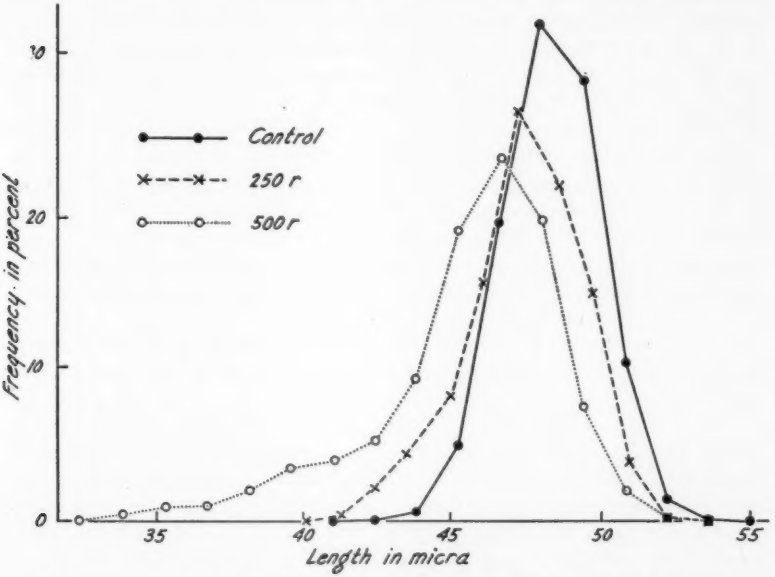

FIGURE 4.—Frequency distributions of length of pollen grain in untreated samples and in samples treated post-meiotically with 250 r and 500 r.

the X-ray treatment causes a reduction in mean length and that most if not all of the increased variability is due to changes in the direction of smaller size. Because of the great natural fluctuations in mean length as witnessed in control data, it cannot be proved that no positive changes were induced. At least it is certain that nearly all these mutations have a negative effect.

It also seems evident from a comparison of these curves that a very large number of pollen grains were affected and likewise that an even greater number of mutations were induced. This must be the case if mutations are distributed at random to all cells, and there is little reason to doubt this. A crude estimate of the number of changes involved may be obtained by fitting to the

treatment curves the control curve modified in various ways. It was found that curves would fit reasonably well only within a rather narrow range of assumptions. For instance, if changes were considered to be distributed equally to a certain percentage of cells and not at random according to Poisson's binomial limit, or if changes were distributed at random, but of equal effect, the resultant curves assumed a shape which was quite different from the treatment curve. The best fit was obtained when 100 changes of varying effect were considered to be distributed at random to every 100 microspores per 200 r. It is appreciated that such empirical approximations are subject to considerable error, yet for the following comparison, they seem useful.

Since the amount of gross chromosomal aberration has been measured in this same material for the same period under consideration, it might be of interest to compare the figure of total gross aberration with the number of mutations derived empirically above. In the studies on chromosomal aberration only deletions, dicentric, and ring chromosomes are detected. The viable types—inversions and translocations—are detectable only in the relatively rare event that they greatly alter the normal arm lengths. There is indirect evidence that these viable or symmetric types are less frequent than the nonviable or asymmetric types to which they correspond (SAX 1940); there seems to be no reason for assuming them to be more frequent. Assuming equality between them, calculations yield the value 39.5 as the total gross chromosomal rearrangements induced per 100 cells per 200 r. This value is far below the mutation number estimated at 100. It is apparent, therefore, that the amount of gross or detectable chromosomal aberration is insufficient to account for the size changes. This discrepancy recalls to mind the disagreement between the dosage curves for chromosomal aberration and pollen abortion noted previously.

The mutation rate necessary for the observed size changes in pollen thus estimated is unusually high. However, no previously determined mutation rates offer a satisfactory comparison. The rate of X-ray induced pollen changes in Zea (STADLER 1941) is very much smaller. Yet since the latter findings depend upon gametophytic transmission, they are hardly comparable. Moreover, Tradescantia is notably more sensitive than Zea in its chromosomal responses to X-ray treatment.

Certainly the relative frequency of size-changing mutations is much greater than that of mutations effecting pollen abortion. For purposes of comparison the latter might be considered as lethal mutations, the former as semi-lethals. A similar instance is to be found in the work of TIMOFÉEFF-RESSOVSKY (1935) on mutations affecting viability in Drosophila. He demonstrated that sex-linked genes which were lethal to only a fraction of the males were two to three times as frequent as those which were lethal to all males. There might conceivably be another and perhaps still larger group of mutants which affect viability to some extent but not so adversely as to be lethal to any individuals. Yet genes of this degree of effect might be the very ones which are responsible for most of the size changes noted in Tradescantia pollen. It was mutation in genes having these slight quantitative effects which BAUR, BRIDGES, and EAST

found to be most frequent in their studies and which they concluded were of greatest importance in evolution.

It seems likely from the preceding evidence that mutations affecting size of pollen as well as those causing pollen abortion are intragenic changes. A familiar alternative is that they are the phenotypic expression of minute chromosomal aberrations, most likely deletions. STADLER (1941) has presented evidence to indicate that all or nearly all the X-ray-induced mutations in contrast to the ultra-violet and spontaneous mutations in Zea are of this deletion or deficiency type. Yet the deletions concerned here must be smaller beyond the optical limit of the compound microscope because the detectable deletions show more of a squared than linear relationship with X-ray dose in Tradescantia (RICK 1940; NEWCOMBE 1942). This is, therefore, another example of the deletion-mutation paradox (cf. RICK 1940), wherein a large proportion of the X-ray-induced mutations are definitely related to chromosomal aberrations; nevertheless, chromosomal aberrations show a squared relationship with dose while mutations show a linear response.

PRE-MEIOTIC TREATMENTS

Meiotic sterility is characterized by a very abrupt rise to nearly complete sterility on the twelfth day after treatment and a very gradual decline in the proportion of aborted pollen during the following period. This decline is very irregular, collections from the same treatment taken on the same day showing a much greater variability than the post-meiotic treatments. Although abortion approaches the normal level, this irregularity persists for two months—that is, for as long a period as the inflorescences continue to produce flowers. This very prolonged sterility obviously cannot be referred exclusively to treatment during meiosis, for a collection taken two months after treatment must trace to a bud in very early developmental stages at the time of treatment. The long duration of sterility might be explained in the following way. Radiation during the development of the archesporium induces many chromosomal aberrations; for example, inversions and translocations, which survive mitosis in diploid cells, but which at meiosis lead to deficiencies and duplications, subsequently to abortion of the gametes. Certain types of aberration such as ring and dicentric chromosomes producing deficiencies immediately might lead to elimination of somatic cells before meiosis, according to SAX (1941). During the very rapid development of the archesporium many nuclei are presumably irradiated in various stages of mitosis. The known variation in sensitivity of chromosomes at different mitotic stages might then account for the observed variation in abortion.

The amount of abortion and the effects on length and variation in length of pollen produced by anthers irradiated during the pre-meiotic period are indicated by the data in table 3. These figures are of uncertain reliability because of the aforementioned fluctuating sterility. In compiling these data it seemed expedient to omit those samples in which pollen abortion was far above the range of most of the other samples. To include such deviating values would produce very skewed sample distributions and, of course, greatly increase hetero-

geneity. Yet, since it is of uncertain reliability to select samples in this manner, it is also uncertain whether the lessened genetic effects noted are real effects; they may merely represent a selection of smaller values. On the other hand, if the genetic effects of radiation are actually smaller during the pre-meiotic period in comparison with the post-meiotic period, this change in sensitivity would not lack a parallel in other studies of X-ray-induced mutation reported in the literature. Evidence of a similar variation in sensitivity in X-ray-induced chromosomal aberrations has been reported by SAX and SWANSON (1941). They found, for instance, that the sensitivity of microspores is of a much higher order than that of root tip cells. It would not be surprising then if the gene sensitivity of the somatic archesporium were lower than that of the microspores. Whether or not the very sterile samples are included in this data, it is certain that the mutational effect in abortion and variation of lengths is induced by the pre-meiotic treatments.

TABLE 3

The effects of X-ray dosage on the mutational response induced in pollen by treatment previous to meiosis.

LOT NO.	DAYS AFTER TREATMENT	TREATMENT	NUMBER OF SAMPLES	POLLEN ABORTION	LENGTH OF POLLEN GRAIN	
					MEAN	VARIANCE
9–25	46 to 50	500 r	10	10.74±0.99%	50.73±0.40μ	1.573±0.228μ²
9–26	46 to 48	250	13	7.91±1.11	50.56±0.26	1.410±0.138
9–28	46 to 48	125	16	6.22±0.83	50.72±0.17	1.216±0.095
9–29	46 to 48	62.5	25	5.35±0.41	50.84±0.12	1.087±0.070
9–30	46 to 48	Control	17	5.15±0.45	50.94±0.13	0.795±0.041

According to tables 2 and 3 the amount of pollen abortion in the untreated controls is significantly higher for the pre-meiotic data than for the post-meiotic data. These collections were taken from the same plants but at two different periods, 35 days apart. This is mentioned only as an example of the unpredictable manner in which pollen formation fluctuates. It complicates the task of securing adequate controls, especially for pre-meiotic studies.

DISCUSSION

It has been demonstrated that X-rays applied to developing microspores even as late as four days after meiosis induce an appreciable genetic effect on size of the microspore. It is perhaps even more striking that there is also a marked increase in the frequency of aborted grains—that is, grains completely lacking cytoplasm. It is of interest in connection with the mutations affecting pollen size to examine the growth curve of normal Tradescantia microspores. This is illustrated in figure 5. It will be noted that most of the increase in length occurs during the maturation following the first microspore mitosis. X-ray treatment during this period has little or no effect, as evidenced by the comparatively smooth horizontal curves obtained for this period (left half of

figures 1 and 2). Treatment during the first mitosis induces a marked rise in effects, especially in pollen abortion, but, as pointed out, these are evidently not of intragenic nature. Thus the mutational effects, if any, of this period are unfortunately obscured. But during the preceding or "critical" period significant genetic effects are induced. Those changes induced by treatment during the earliest part of this period might possibly be of greater effect, but if this trend does exist, it is relatively slight. More significant is the fact that these changes seem to be induced during this period and gain expression after mitosis during the period of more rapid cell elongation.

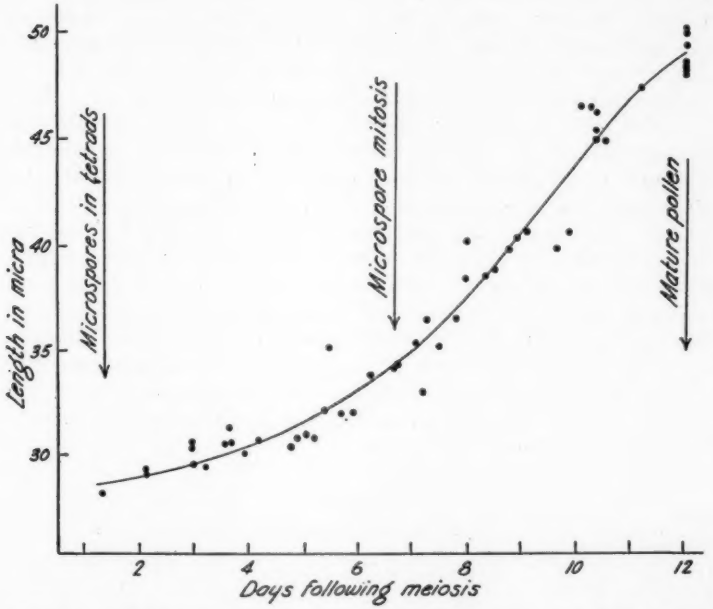

FIGURE 5.—Elongation of microspores in relation to time following meiosis. Each point represents the mean of a sample of 25 microspores. The curve indicated is a freehand fit to the sample means.

Genetic control of size of the microspore was discovered by SATINA and BLAKESLEE (1937) in triploid Datura and by SAX (1937) in triploid Tradescantia to be expressed as early as the time of microspore mitosis. In comparing size of the microspore with chromosome number at the microspore mitosis, they found variability in size to increase with increasing unbalance of the chromosome complement. Variation in amount of chromatin was insufficient to account for this variability in size. Apparently different chromosomes exert a differential influence on size, and this control would be most variable where the greatest number of different chromosome combinations can occur—in the more unbalanced microspores. In the case of these triploids, however, number

of chromosomes per microspore is determined at the first meiotic division. Genetic control of size might produce its phenotypic effects at the time of the second meiotic division. It is conceivable, furthermore, in these instances, that the cytokinesis which occurs at telophase of the first meiotic division might apportion the amount of cytoplasm of the pollen mother cell to each daughter cell in accordance with the number and kind of chromosomes in the telophase nuclei. This type of control is evident in pollen mother cells in which univalents or other lagging chromosomal associations sometimes develop into micronuclei and are surrounded at cytokinesis by a correspondingly small amount of cytoplasm in the formation of diminutive, though functionless microspores. A study of the development of small pollen in heterozygotes of the *sp* genes in Zea (MANGELSDORF 1932; RHOADES and RHOADES 1939) might be interesting in this regard.

In the present experiment it seems that either mutations affecting size and abortion of pollen require the completion of mitosis before they gain phenotypic expression, or, as suggested by STERN (1938), the resting stage following the microspore mitosis might not be long enough to permit mutations induced during this period to gain expression, any possible concurrence of phenotypic expression and mitosis in later development therefore being only coincidental. Another perhaps more likely explanation relates to the nuclear condition of the post-mitotic microspore. Any recessive mutation induced subsequent to the stage at which chromosomes are effectively split into chromatids in respect to X-ray action could not be expressed because of the presence of its dominant allele in the same cell. Obviously this masking effect could occur only if both the tube nucleus and generative nucleus exert a controlling influence on pollen development. They differ in appearance and staining reaction, yet both continue to function, at least until anthesis.

SUMMARY

X-rays were applied to plants of Tradescantia in such a manner that inflorescences received the doses 62.5, 125, 250, and 500 r. By examining buds and timing the rate of flowering, the time elapsed during various periods of the nuclear cycle in development of the microspore was determined. Knowing the lengths of these periods, it was then possible to irradiate during certain stages of development by treating inflorescences and later collecting pollen at the required time after treatment.

Length of pollen grain and percentage of aborted grains were determined in the subsequently developed pollen. Much of this data was characterized by internal heterogeneity, which, on the application of appropriate tests, proved to be significant. In order to make statistical comparisons between lots, this difficulty was overcome by taking many samples of small size.

The daily fluctuations of percentage of pollen abortion, mean pollen length, and variance of length were observed over a two month period following treatment. When microspores were treated during the period from meiosis to the first mitosis, pollen abortion subsequently rose to a significantly higher level than controls; mean lengths decreased and variance of length increased sig-

nificantly. It has been demonstrated previously that these are mutational responses.

These mutational responses increase with increasing X-ray dose. The response of pollen abortion is approximately linear while that of variance of length is intermediate between a linear and squared relationship.

The changes of length induced by the treatment are all or nearly all in the negative direction. Comparisons of curves yield a crude estimate of 100 size changes or mutations per 100 grains per 200 r. This greatly exceeds the amount of gross chromosomal aberration, approximately 40 per 100 cells, induced by the same dose in the same material.

The linear response to dosage in pollen abortion and the nature of the induced size changes bear close resemblance to X-ray effects in other organisms which have been proved to be the result of mutation. This similarity is considered further evidence that the changes in pollen are the phenotypic expression of mutations induced by the X-ray treatment.

A rise to nearly complete pollen abortion occurred after X-rays had been administered during meiosis and the preceding period of development. A secondary peak of lower level was found to correspond to the first gametophytic mitosis. According to evidence presented, these rises in sterility are not strictly of the gene mutational type. They are more likely related to chromosomal aberration.

The same measurements were made after subsidence of meiotic sterility, approximately 50 days after treatment. The very irregular return to a nearly normal level of pollen abortion renders these measurements uncertain. Nevertheless, the mutational responses also seem to occur when X-rays are thus applied during the pre-meiotic period.

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INHERITANCE AND PHYSIOLOGY OF EFFICIENCY IN IRON UTILIZATION IN SOYBEANS[1]

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INTRODUCTION

STRIKING differences in chlorosis typical of iron deficiency were noted in 1938 among a considerable number of soybean varieties when tested on calcareous soils for the first time since their introduction into the United States from Manchuria. Because these strains were not greatly different morphologically, it was thought that such wide differences in chlorosis would lend themselves especially well to a study of the inheritance and physiology of iron availability in plants.

Reported cases of significant differential responses of diverse plant genotypes within the same species to varying nutrient levels are not numerous. The more important to date have been reviewed recently by BURKHOLDER and MCVEIGH (1940) and will not be discussed here. They largely concern differential response of inbred lines and hybrids of corn to varying increments or sources of the major growth elements.

No previous report has been found of intraspecific heritable differences in the hydrogen-ion concentration of vegetable plant tissues. Genetically controlled differences in the acidity of flower petal sap of *Primula sinensis* and other species have been reported by SCOTT-MONCRIEFF (1936, 1939). Macerated petal tissue of plants homozygous for the single recessive gene causing low acidity showed a pH of approximately 6.0 while the dominant allele resulted in a flower petal pH of approximately 5.3.

Although numerous investigations have been conducted on the availability of iron to plants, the problem is still unsolved in several aspects. Chlorosis due to iron deficiency has frequently been reported when plants were grown in either soils containing an excess of lime or nutrient solutions with high pH. Most early investigators assumed that the low hydrogen-ion concentration in either case caused iron to be precipitated in the medium, thereby rendering it non-available to plants. Discoveries that in many cases chlorotic tissues contained as much or more iron than normal tissues have altered the concepts of iron availability. Since iron may occur in abundance in tissues of chlorotic plants, it would seem that much or all of this iron is in non-available form, suggesting that some internal factors may be involved in its availability to chlorophyll-containing cells.

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Growth of *Zea mays* and other species, when plotted against pH of full nutrient solution media to which iron had been added in an inorganic form, was found by OLSEN (1935) to follow a bi-modal curve. Plants grown in solutions held at pH 4.5 or 8 developed no chlorosis, but those grown in solutions of pH 6 or 7 exhibited severe iron deficiency symptoms. Solution analyses revealed decreasing soluble iron with increasing pH until a minimum was encountered at pH 6. Corn tissue analyses revealed larger amounts of iron in chlorotic (pH 6 and 7) than in normal cultures (pH 4.5 and 8). OLSEN concluded that although adequate iron was absorbed in all cultures, at pH 6 and 7 considerable phosphate ions were also absorbed, resulting in precipitation of iron as ferric phosphate within the vascular bundles. At pH 8 a large portion of the phosphate was precipitated in the solution as calcium phosphate, thereby rendering phosphate concentration low in the tracheal sap resulting in adequate availability of iron. OLSEN contended that at pH 4 and 5, corn plants normally absorb less phosphate than at a higher pH, and, furthermore, as numerous investigations have shown that pH of the growth medium influences pH of cell sap to some extent, the sap was adequately high in hydrogen-ion concentration to prevent precipitation of iron. Later work supported these conclusions. No chlorisis occurred in media with pH from 4 to 8 when phosphate was restricted. With abnormally high phosphate, chlorosis occurred at the highest pH as well as at pH 6 and 7. OLSEN surmised that in the latter case phosphate was added in excess of calcium in the solution which permitted absorption of adequate quantities to precipitate the iron in the tracheal vessels. Low calcium content of plants grown under these conditions substantiated this viewpoint.

MATERIALS AND METHODS

Source of genetic material

From approximately 20 soybean introductions from the Orient by the DIVISION OF PLANT EXPLORATION AND INTRODUCTION, U. S. DEPT. OF AGRICULTURE, that exhibited iron deficiency symptoms, six were selected for this study. The efficient types were pure lines of four standard varieties that exhibited chlorosis only on soils containing above 40 percent calcium carbonate.

Crosses were made reciprocally in all possible combinations in 1939 and 1940 between and among the four efficient and six inefficient varieties, and backcrosses were made to the inefficient parents. The F_1, F_2, F_3, and backcross generations of these crosses were grown in the greenhouse and in the field and the classification of their efficiency in iron utilization determined in a special nutrient solution described below.

Media for genotype classification

Development of a growth medium in which proper concentration of available iron could be maintained to give the differential response exhibited on calcareous soils proved difficult. Classification of efficiency types was not found feasible in highly calcareous soils in the greenhouse during the period of low light intensity encountered throughout the winter. Deposition of calcium carbonate in high lime soils of Iowa is highly variable, and no degree of hand mix-

ing rendered this soil sufficiently homogeneous in available iron to permit classification in the early stages of development.

Use of quartz gravel, sub-irrigated with nutrient solution, was attempted. Neither partial removal with acids of iron naturally present in crushed quartz nor complete removal with subsequent addition of magnetite proved suitable under the conditions of these experiments.

Nutrient solutions seemed to offer greater homogeneity than any other growth medium. In full nutrient solutions, however, adequate amounts of available iron could not be maintained for normal growth of even efficient varieties without maintaining the solution at a pH well below 6. Since nutrient absorption caused the pH of solutions to fluctuate greatly, this method was considered impractical.

Preliminary studies had shown that when phosphorus was restricted in the nutrient solution no iron deficiency symptoms occurred even though the pH remained at approximately 7.4. Furthermore, it was found that when an excess of iron tartrate was added to the solution, phosphate could be added in increasing amounts until the concentration of available iron was such that the iron efficient varieties were normal in every respect while the inefficient varieties exhibited severe chlorosis at the first trifoliate leaf stage. To maintain this differentiating level of available iron it was necessary to add small quantities of phosphate to the solution every few days. If no additional phosphate was added, the chlorotic plants slowly developed chlorophyll presumably because absorption of the phosphates in the solution rendered additional iron available. Since the differentiating level could be maintained readily, this medium was chosen for classification of genotypes. SMITH'S (1934) nutrient solution was used minus KH_2PO_4. Approximately one part per million of iron was added in the form of ferric tartrate. An initial addition of slightly more than one part per million of the phosphate ion established the desired differentiating level of available iron. The solution was replenished with approximately one-fifth part per million of phosphate every two days to maintain the desired level of available iron.

Classification of genetic material

Seeds for the F_1, F_2, F_3, backcrosses, and parents were germinated in gravel, and, after emergence, the seedlings were transferred to nutrient solution tanks. The extent of differentiation in chlorosis among the parents was used as a criterion for establishing the appropriate level of available iron. All material was inspected at least three times at two-day intervals and classified according to iron deficiency symptoms as evidenced by chlorosis. Each plant was assigned a score ranging from zero (dark green) to five (bleached yellow appearance to complete necrosis of the leaf and death of the growing point).

Sampling for compositional differences between parents

In the study of compositional differences between the parents it was considered advisable to grow these varieties in nutrient media under conditions which would promote optimum growth for both types. A moderate degree of

chlorosis was allowed to develop in the first trifoliate leaf of the inefficient varieties to prove their genetic purity. When approximately 4 ppm of iron in the tartrate form was supplied, all plants developed normal chlorophyll, and subsequent leaves showed no iron deficiency symptoms. Phosphate was added frequently in very small quantities, and it was presumed an adequate amount was available, since no plants exhibited nutrient deficiencies.

When the plants attained a height of approximately 14 inches two replications were removed from the tank, and the four plants of each variety, which constituted an experimental unit, were separated into stem and leaf samples. The leaves were rapidly divided into two samples by cutting each leaflet longitudinally with nickel plated shears. Stems were cut into one-half-inch lengths, mixed thoroughly, and divided into two samples. One of the two leaf and stem samples was immediately placed in a small, tightly stoppered vial and frozen on a layer of dry ice. The other sample was dried at 125°C.

Several hours later the frozen samples were removed as needed, the vials immersed in tepid water for several minutes, and the composite tissue fluid extracted in a small plant press constructed of low-iron brass. The fluid was caught in a glass vial and the desired amount transferred to the small cup of a glass electrode attachment to a Coleman 3C pH electrometer suitable for making determinations in micro-quantities of liquids. After the pH was determined, one ml of the filtered fluid, or a known portion of one ml, was pipetted into a Pyrex test tube for subsequent determination of soluble iron.

The oven dried samples were ground with a mortar and pestle, again placed in the drying chamber for several days, and stored in a desiccator until total iron determinations were made.

The remaining two replications in the nutrient solution tank were harvested two weeks later and prepared for analysis as previously described.

Analytical procedure

The tissue fluids (1 ml) and dry tissue samples (0.5 gm) were analyzed for iron using the o-phenanthroline method as described by SAYWELL and CUNNINGHAM (1937). Deviations from their technique consisted only in choice of acids for wet ashing, method of determining color intensity, and o-phenanthroline solvent used. One ml concentrated H_2SO_4 and one-half ml concentrated HNO_3 were used for digestion of organic matter. All excess HNO_3 was removed by boiling. Reduction of the ferric iron was accomplished with an excess of hydroxylamin-hydrochloride. One ml 0.10 percent aqueous solution of o-phenanthroline was subsequently added. The sample was then diluted with two ml of distilled water to diminish the violence of subsequent neutralization with concentrated NH_4OH. Congo red paper was used as an indicator. The solution was made up to 10 ml volume in the case of stem samples and 50 ml volume in the case of leaf samples.

Intensity of the red ferrous o-phenanthroline complex was measured with an Evelyn photo-electric colorimeter. A 510 filter was used which had a transmission maximum at approximately 7080 A°, found to be the maximum absorption band of the ferrous o-phenanthroline complex by FORTUNE and MELLON

(1938). The absorption curve was determined by galvanometer readings for standard iron solutions of various concentrations when plotted against parts per million of iron. This enabled direct transposition of galvanometer readings of samples with unknown iron content to parts per million.

The ground, dried leaf tissue was also analyzed for total potassium. The procedure employed was the modified sodium cobaltinitrite method as described by BROWN, ROBINSON, and BROWNING (1938). Sample size and method of digestion were the same as used for the determination of total iron. Percentage of potassium was calculated on a dry weight basis.

RESULTS

Parental differentiation on calcareous soil

To test the magnitude of differential performance of the five efficient and six inefficient varieties, four seedlings of each variety were transplanted into

TABLE 1

Analysis of variance of dry top weights of four efficient and six inefficient soybean varieties when grown in normal and calcareous soil.

SOURCE OF VARIATION	D F	MEAN SQUARES	
		NORMAL SOIL	CALCAREOUS SOIL
Reps.	2	6.89	0.07
Varieties:			
Eff. vs. Ineff.	1	9.19	34.35*
Within Eff.	3	4.20	0.33
Within Ineff.	5	2.20	0.25
Error	18	2.302	0.188

* F value exceeds the 1 percent point in level of significance.

pots of normal soil and soil obtained from a naturally occurring calcareous area containing approximately 30 percent calcium carbonate and having a pH of approximately 7.3. These treatments were replicated three times. Extreme chlorosis occurred in the inefficient plants when grown on the high carbonate soil, while no deficiency symptoms occurred in the efficient genotypes.

The analysis of variance for dry weight of tops at four weeks after transplanting is given in table 1. Differences within the efficient and the inefficient varieties on either calcareous or normal soils were not significant. Although on normal soils the mean dry weights of the two groups were not significantly different, the differences when grown on calcareous soil were highly significant.

Inheritance of efficiency in iron utilization

As previously described, genotype classification in the inheritance studies was based on the degree of chlorosis exhibited by the seedlings when grown in a nutrient solution which had been rendered low in available iron.

Although the testing of progenies extended throughout periods of low and adequate light intensities, as encountered throughout the winter and spring seasons, overlapping of classification of the efficient and inefficient parents occurred infrequently. Therefore, all tests of plants with comparable genetic constitution were grouped regardless of the period of testing.

Performance of parents

The performance of all parental varieties which were included in the various tests is summarized in table 2. The average chlorosis grade of the efficient parents was 0.09, while the mean of the inefficient parents was 3.78. Only one plant of the efficient parents was assigned a chlorosis grade above 1, and only 8 inefficient parent plants were given a grade below 2. Consequently, if the genotypes of these plants had not been known, and if all plants assigned chlorosis grades of 0 and 1 had been considered efficient genotypes and plants

TABLE 2

Summary of performance of efficient and inefficient parents when tested on low-available-iron media.

VARIETY	CHLOROSIS GRADE						MEAN GRADE
	0	1	2	3	4	5	
Efficient:							
1 (Dunfield)	26	—	—	—	—	—	0.0
2 (Mandell)	20	3	1	—	—	—	0.2
3 (Illini)	30	2	—	—	—	—	0.1
4 (Mukden)	38	4	—	—	—	—	0.1
Total	114	9	1	—	—	—	0.09
Inefficient:							
5 (FPI. 54619-5-1)	—	—	3	1	8	19	4.4
6 (FPI. 88508)	—	—	3	7	9	2	3.5
7 (FPI. 88358)	—	—	—	—	6	9	4.6
8 (FPI. 88294)	—	2	3	20	19	25	3.9
9 (FPI. 87617)	—	4	9	18	24	15	3.5
0 (FPI. 88354)	—	2	7	8	7	6	3.3
Total	—	8	25	54	73	76	3.78

assigned grades of 2 to 5 inefficient genotypes, nine plants out of 360, or one out of 40 would have been classified erroneously. The frequency of error in genotype classification in segregating progenies was probably of this magnitude.

Performance of F_1 crosses

The frequency distribution of F_1 crosses within the six chlorosis grades in comparison with that of the parents appears in table 3. In this classification grades 0 to 1 included plants in which no definite iron chlorosis appeared, and grades 2 to 5 included plants showing different degrees of iron deficiency symp-

TABLE 3

Frequency distribution of chlorosis grades of parents and various F_1 crosses when grown in media low in available iron.

GENETIC MATERIAL STUDIED	CHLOROSIS GRADES							
	0	1	TOTAL 0 TO 1	2	3	4	5	2 TO 5
Eff. Parents	114	9	123	1	—	—	—	1
Ineff. Parents	—	8	8	25	54	73	76	228
Eff.×Eff. F_1	44	3	47	—	—	—	—	0
Ineff.×Ineff. F_1	—	1	1	6	14	23	86	129
Ineff.×Eff. F_1	87	5	92	—	—	—	—	0

TABLE 4

Numbers of efficient and inefficient plants in F_2 populations resulting from crosses between efficient and inefficient varieties.

CROSS	EFFICIENT	INEFFICIENT	CHI-SQUARE* (3:1)
1×5	45	14	0.05
1×6	37	17	1.21
1×7	38	21	3.53
1×8	40	15	0.15
1×9	41	12	0.16
1×0	33	12	0.07
2×5	38	16	0.65
2×6	38	15	0.31
2×7	26	9	0.01
2×8	38	14	0.10
2×9	36	10	0.26
2×0	43	10	1.06
3×5	40	15	0.15
3×6	44	10	1.21
3×7	43	14	0.01
3×8	41	14	0.01
3×9	41	14	0.01
3×0	41	12	0.16
4×5	21	8	0.10
4×6	30	12	0.29
4×7	35	11	0.03
4×8	26	13	1.44
4×9	38	15	0.31
4×0	13	4	0.02
Total all F_2	886	305	0.69

* χ^2 for 1 D F at 5 percent level = 3.84.

toms. The frequency distributions have been grouped into these two major classes.

In all six efficient×efficient crosses only efficient F_1 plants were obtained. The 14 F_1 crosses of inefficient×inefficient types resulted only in inefficient progeny, indicating that all the inefficient varieties possessed a common gene or genes conditioning the inefficiency in iron utilization. F_1 plants in each of 14 crosses between inefficient and efficient varieties were as efficient in iron utilization as their efficient parents, indicating complete dominance of this type. The dominance of iron efficiency under field conditions on calcareous soil is illustrated in figure 3.

Performance of efficient×inefficient F_2 populations

F_2 populations of all possible crosses between the four efficient and six inefficient parents were tested in a nutrient solution rendered low in available iron (table 4). The ratio of efficient to inefficient plants in the total for all F_2 populations did not deviate significantly from the ratio of a 3:1 hypothesis. Each cross was also tested for conformity to this ratio by the chi-square test and revealed close agreement. The difference between the four efficient and six inefficient varieties in efficiency of iron utilization seemed to be conditioned by a single major gene.

Performance of backcross populations

F_1 plants from five crosses between two inefficient and three efficient parents were backcrossed to their respective inefficient parents. The frequency distributions of the backcross populations in regard to iron utilization efficiency appear in table 5.

TABLE 5

Numbers of efficient and inefficient plants in backcross populations.

BACKCROSS	EFFICIENT	INEFFICIENT	CHI-SQUARE* (1:1)
8×(8×1)	13	12	0.04
8×(8×2)	32	29	0.02
8×(8×3)	7	13	1.80
Total χ^2			1.86
Total plants	52	54	0.04
9×(1×9)	24	22	0.09
9×(3×9)	14	10	0.67
Total χ^2			0.75
Total plants	38	32	0.51
Total	90	86	0.09

* χ^2 for 1 D F at 5 percent level = 3.84.
χ^2 for 2 D F at 5 percent level = 5.99.
χ^2 for 3 D F at 5 percent level = 7.81.

None of the backcross populations differed significantly from a 1:1 ratio.

FIGURE 1.—Four efficient (left four pots) and six inefficient (right six pots) soybean varieties growing in soil with low available iron.

FIGURE 2.—Four efficient (left four pots) and six inefficient (right six pots) soybean varieties growing in soil with adequate available iron.

FIGURE 3.—Three plants of parent 8 (left), three 8×4 F_1 plants, and two plants of parent 4 when growing on calcareous soil.

FIGURE 4.—Three F_3 lines when grown in low-available-iron nutrient solution—homozygous inefficient (upper row), segregating (center row) and homozygous efficient (lower row).

Performance of F_3 lines

During the summer of 1940, F_2 populations from efficient×inefficient crosses involving efficient parents 2 and 4, and inefficient parents 6, 8, 9, and 0, were grown to maturity in soil with adequate available iron. The progeny of a limited number of F_2 plants, selected at random from each cross, were tested to determine their efficiency in iron utilization when grown in nutrient solution containing a low level of available iron. Because of limited facilities, only ten F_3 plants were grown as a test of each F_2 individual. If a single factor difference is assumed, this small number of plants could result in erroneous classification of approximately seven percent of the segregating progenies. Performance of F_3 progenies appears in table 6. A segregating line grown by chance between apparently homozygous efficient and inefficient lines is shown in figure 4.

TABLE 6

Classification of efficient×inefficient F_2 populations on the basis of their progeny performance in the F_3 generation when grown in media low in available iron.

CROSS	HOMOZYGOUS EFFICIENT	SEGREGATING	HOMOZYGOUS INEFFICIENT	CHI-SQUARE* (1:2:1)
6×2	2	9	5	1.37
8×2	3	7	7	2.41
9×2	3	9	6	1.00
0×2	3	8	5	0.50
4×6	2	10	6	2.00
4×8	6	5	4	2.20
4×9	2	11	3	2.37
4×0	3	10	5	0.67
Total χ^2				12.52
Total plants	24	69	41	4.43

* χ^2 for 2 D F at 5 percent level = 5.99.
χ^2 for 16 D F at 5 percent level = 26.30.

The proportion of homozygous efficient, segregating, and homozygous inefficient F_3 lines in none of the F_2 populations deviated significantly from the expected 1:2:1 ratio. Furthermore, the sum of the chi-square values for all populations, and the chi-square value calculated from the deviations of total plants in each class from the expected number were also non-significant. Distribution of types in the segregating lines is shown in table 7.

None of the population totals for segregating lines deviated significantly from the 3:1 ratio. The total chi-square, as well as the chi-square for the ratio of all efficient and inefficient plants in the segregating lines, also indicates conformity with the single factor hypothesis.

Maternal inheritance test

Since reciprocal crosses between efficient and inefficient parents were frequently made, the data were examined as to the possibility of maternal inheritance (table 8).

TABLE 7

Numbers of efficient and inefficient plants in F_2 segregating lines.

CROSS	EFFICIENT	INEFFICIENT	CHI-SQUARE* (3:1)
6×2	66	26	0.52
8×2	47	19	0.51
9×2	63	24	0.31
o×2	56	23	0.71
4×6	70	29	0.97
4×8	34	16	1.31
4×9	79	31	0.59
4×o	75	24	0.03
Total χ^2			4.95
Total plants	490	192	3.61

* χ^2 for 1 D F at 5 percent level = 3.84.
χ^2 for 8 D F at 5 percent level = 15.51.

TABLE 8

Performance of F_1 and F_2 generations of reciprocal crosses.

TYPE OF PARENT			CHLOROSIS GRADES								CHI-SQUARE* (3:1)
FEMALE		MALE	o	1	TOTAL EFF.	2	3	4	5	TOTAL INEFF.	
Ineff.	×	Eff. F_1	60	3	63	—	—	—	—	o	—
Eff.	×	Ineff. F_1	42	2	44	—	—	—	—	o	—
Ineff.	×	Eff. F_2	302	45	347	14	48	33	38	133	1.74
Eff.	×	Ineff. F_2	451	68	519	26	62	51	34	173	0.00

* χ^2 for 1 D F at 5 percent level = 3.84.

Absence of maternal inheritance was evidenced by the similarity in performance of reciprocal crosses in both the F_1 and F_2 generations.

Physiological study

Leaf and stem tissues of the four efficient and six inefficient varieties used in the inheritance study were subjected to certain chemical analyses as previously outlined.

The following analyses were made: (1) pH of composite tissue fluid expressed after freezing samples of leaves and stems, (2) soluble iron in one ml of composite tissue fluid from leaves and stems, (3) total iron in dried stem and leaf tissues, and (4) total potassium in dried leaf tissues. Mean values of the four replications for the four efficient and six inefficient varieties appear in table 9.

The criteria, for which means are presented in table 9, have been subjected to analysis of variance (table 10).

TABLE 9

Mean pH and soluble iron values of composite tissue fluids, soluble and total iron of leaf and stem tissues, and total potassium of leaf tissues (dry weight basis) of four efficient and six inefficient soybean varieties.

VARIETY	LEAVES				STEMS		
	pH	SOLUBLE Fe (ppm)	TOTAL Fe (%)	TOTAL K (%)	pH	SOLUBLE Fe (ppm)	TOTAL Fe (%)
Efficient:							
1	6.27	6.28	.098	3.56	5.90	5.70	.016
2	6.20	6.70	.096	3.75	5.88	5.10	.015
3	6.18	7.50	.093	3.90	5.87	5.08	.014
4	6.12	6.53	.085	4.04	5.83	6.45	.015
Mean	6.19	6.75	.093	3.81	5.87	5.83	.015
Inefficient:							
5	6.51	4.15	.121	3.48	5.99	5.93	.019
6	6.43	4.98	.110	3.48	5.98	5.00	.019
7	6.37	6.73	.097	3.70	5.96	6.10	.016
8	6.50	5.58	.133	3.39	5.99	5.50	.017
9	6.37	6.20	.101	3.58	5.92	6.15	.017
0	6.43	5.68	.105	3.52	5.95	4.98	.018
Mean	6.43	5.68	.111	3.53	5.97	5.61	.018

TABLE 10

Analysis of variance of pH, soluble iron, and total iron of leaf and stem tissues, and total potassium of leaf tissue of ten soybean varieties.

SOURCE OF VARIATION	D F	MEAN SQUARES			
		pH	SOLUBLE IRON	TOTAL IRON	TOTAL POTASSIUM
Leaves:					
Replications	3	.0034	0.10	.000264	.1425
Varieties					
Eff. and Ineff.	1	.5520†	11.10*	.003271*	.8109†
Within Eff.	3	.0164	1.12	.000126	.1668
Within Ineff.	5	.0151*	3.81	.000742†	.0438
Error	27	.00584	1.519	.0001740	.09022
Stems:					
Replications	3	.0006	0.32	.000000	
Varieties:					
Eff. and Ineff.	1	.0920†	0.48	.0000616†	
Within Eff.	3	.0039*	1.39	.0000033	
Within Ineff.	5	.0028	1.14	.0000038*	
Error	27	.00118	0.810	.00000133	

* F value exceeds the 5 percent point in level of significance.
† F value exceeds the 1 percent point in level of significance.

The analysis of variance shows that efficient and inefficient varieties differed significantly in hydrogen-ion concentration of composite tissue fluid of both leaves and stems. Although significant differences were found in the pH of leaf tissue among the inefficient, and in the pH of stems among the efficient varieties, these differences were far less in magnitude than differences between the efficient and inefficient varieties. The F values for leaves and stems of the latter were, respectively, 12 and 10 times the value necessary for highly significant differences.

These differences were substantiated by pH determinations of composite leaf tissue fluid of the ten varieties when grown in the field on non-calcareous soil during the summer of 1940. Under these conditions the F value of the pH difference between the two types exceeded that of the one percent point in level of significance by five times.

Differences in total iron content of tissues closely paralleled differences in pH of tissue fluid. Inefficient varieties contained a surprisingly greater amount of total iron than the efficient varieties. Analyses for soluble iron showed a significantly larger quantity in the expressed sap of leaf tissue from the efficient type. Although soluble iron in the stems showed the same relationship, the differences were statistically non-significant. Total potassium was significantly greater in the efficient varieties, while varieties within the two groups did not differ significantly.

The gene conditioning greater efficiency in iron utilization thus seems to be expressed by its action in producing higher hydrogen ion concentration, higher soluble iron, higher potassium and lower total iron content of aerial tissues than its allele which, when in the homozygous state, conditions inefficiency in iron utilization. A study of the associations between these various compositional criteria was made by means of the covariance method of analysis as outlined by SNEDECOR (1940). The relative degree of association was considered a possible means of approaching the cause and effect relationship. The following associations in the leaf tissues were investigated: pH and soluble iron, potassium and soluble iron, pH and total iron, potassium and total iron, and pH and potassium. The stem analysis associations studied were pH and soluble iron, and pH and total iron. The resulting errors of estimate mean squares and adjusted mean values for the efficient and inefficient varieties appear in table 11. Mean squares from the analysis of variance and unadjusted mean values for the two types of varieties are included to facilitate comparison of adjusted and unadjusted values.

The mean squares of errors of estimate indicate a close association between the hydrogen iron concentration of the expressed juice and total iron within the leaf and stem tissues. Highly significant total iron differences between the efficient and inefficient varieties were rendered non-significant after due allowance was made for pH differences.

Similarly, potassium and soluble iron differences within leaf tissues became non-significant when pH variation was taken into account. Although adjustment for the potassium association did reduce soluble and total iron differences between the efficient and inefficient genotypes, the potassium content did not

TABLE 11

Mean squares of errors of estimate and adjusted mean values of efficient and inefficient varieties resulting from the analysis of covariance of various compositional criteria.

SOURCE OF VARIATION	D F	SOLUBLE IRON			TOTAL IRON			TOTAL K	
		UNADJ.	ADJ. FOR pH	ADJ. FOR K	UNADJ.	ADJ. FOR pH	ADJ. FOR K	UNADJ.	ADJ. FOR pH
Leaf Tissues:									
Varieties:									
Eff. and Ineff.	1	11.10*	0.23	3.02	.003271†	.000247	.000798*	.8109†	.0024
Within Eff.	3	1.12	1.21	0.95	.000126	.000034	.000022	.1668	.0445
Within Ineff.	5	3.81	2.02	3.05	.000742	.000168	.000475†	.0438	.0140
Error	27‡	1.519	1.372	1.38	.0001740	.000091	.000111	.09022	.08263
Variety Means:									
Efficient		6.75	5.91	6.49	.0928	.1103	.0975	3.81	3.62
Inefficient		5.68	6.24	5.85	.1112	.0995	.1080	3.52	3.65
Difference		1.07	−0.33	0.64	−.0184	.0108	−.0105	0.29	−0.03
Stem Tissues:									
Varieties:									
Eff. and Ineff.	1	0.48	1.04		.00006161	.0000013			
Within Eff.	3	1.39	0.78		.0000030	.0000014			
Within Ineff.	5	1.14	1.02		.0000039*	.00000391			
Error	27‡	0.810	0.743		.00000133	.00000097			
Variety Means:									
Efficient		5.83	5.30		.0152	.0162			
Inefficient		5.61	5.97		.0177	.0170			
Difference		0.22	−0.67		−.0025	−.0008			

* F value exceeds 5 percent point in level of significance.
† F value exceeds 1 percent point in level of significance.
‡ 26 D F for mean squares of errors of estimate.

seem to account for as much of the iron variation as was explained by the hydrogen-ion concentration differences.

DISCUSSION

In view of the complex nature of mineral absorption and utilization in plants, the monogenic simplicity of the genetic control of iron utilization efficiency discovered in these investigations seems remarkable. Although some variation of efficiency was noted among the inefficient varieties, the magnitude of expression of any modifying genes was negligible in comparison with that of the major gene involved. Designation of the recessive gene conditioning inefficiency in iron utilization by the symbol *fe* is suggested. The dominant allele conditioning efficiency would therefore be *Fe*.

Since the gene conditioning efficiency proved identical in the six varieties, and since 14 additional inefficient varieties probably carried this gene, an effort was made to trace the origin of varieties of this type. These varieties came from central and southern Manchuria or northern Chosen. Several of the inefficient varieties were collected from the same locality, but in all cases efficient varieties were also obtained from the same locality. No marked selection against the inefficient genotype seems to have occurred. This is especially odd since calcareous and alkaline soils of various kinds have been found by THORP (1936) to occur generally throughout central and southern Manchuria.

Since soybean varieties in the Orient are generally developed for local areas and normally do not spread rapidly, it is possible that the inefficient varieties were grown on upland soil from which lime had been leached, and no selection against this genotype occurred.

Chemical analyses of stem and leaf tissues were made to determine the physiological differentiation between the genotypes. Differences in hydrogen-ion concentration of expressed tissue fluid were most consistent of the constituents measured. Such differences were obtained not only when the two genotypes were grown in nutrient solutions but also when grown on normal soil under field conditions. The identification of the hydrogen-ion concentration of expressed cell sap with the true intracellular pH is open to criticism. As pointed out by OSTERHOUT (1922), expressed juice does not represent an unaltered cell sap, for, in crushing the cells, the sap may be changed by chemical reactions, absorption, or admixture with intercellular substances. ROGERS and SHIVE (1932) found that although various tissues within a plant differed greatly in hydrogen-ion concentration, the pH of expressed fluid was fairly constant and approximated the mean hydrogen-ion concentration of the various tissues. Furthermore, the pH of composite tissue fluid of various species was proportionate to the pH of similar tissues within the different species. The observed pH difference of the efficient and inefficient soybean varieties is therefore believed to be proportional to the intracellular pH difference of the two types.

Relatively lower solubility of the iron and lower potassium content was associated with the higher pH of the inefficient genotype. The interrelation of cell sap pH, total and soluble iron, and potassium content of plant tissues is not well understood although various associations have been reported. The investigations of INGALLS and SHIVE (1931) and ROGERS and SHIVE (1932) showed a definite relationship between the pH of expressed tissue fluid and the solubility and quantity of iron present within the plant.

HOFFER and TROST (1923) contended that excessive accumulations of iron in the nodal regions of the corn stem were often associated with inadequate supplies of available potassium in the growth medium, and consequently, in the corn stems. The converse interrelation of potassium and calcium absorption by plants, as found by LOEHWING (1928), may be of significance. Media with high calcium resulted in high sap pH and copious precipitation of iron in root and lower stem regions, while a consistent decrease of pH in the expressed plant sap was associated with the addition of potassium to the growth medium. This relationship was considered responsible for a lack of iron deficiency symptoms accompanying the potassium treatment.

In view of these various findings it is suggested that the inefficient gene may condition lower potassium concentration which produces higher pH of cell sap and thereby causes the solubility of iron to be decreased. Although in this study potassium analyses were conducted as an afterthought when genotype differences in iron solubility were discovered, an investigation is planned to ascertain the comparable efficiency of the two genotypes when grown on various levels of potassium. The results of such an experiment coupled with tissue

analyses of iron and potassium should give further information on this complex cause-and-effect relationship.

SUMMARY

Marked differences in chlorosis were noted among soybean varieties when tested on calcareous soils for the first time since their introduction into the United States from Manchuria. Testing of these varieties in nutrient solution cultures and in sub-irrigated crushed quartz media proved that this differential performance could be induced when the plants were grown on media in which the concentration of available iron was low. When grown on such media, varieties which were efficient in iron utilization made normal, green growth, while inefficient varieties showed severe chlorosis symptomatic of iron deficiency which ultimately resulted in death of the plant. The difference between efficient and inefficient genotypes was of sufficient magnitude to permit classification when grown in media rendered low in available iron, which made possible a study of the mode of inheritance of efficiency differences.

On the basis of F_2 and F_3 populations of crosses between four efficient and six inefficient varieties, and of backcross populations, differences in efficiency of iron utilization were shown to be conditioned by a single gene. Inefficiency of F_1 plants from all crosses among the six inefficient varieties established allelism of the gene conditioning inefficiency, and the assumption was made that the inefficient gene was identical in these varieties. The recessive gene conditioning inefficiency in iron utilization was assigned the symbol *fe*.

Performance of F_1 plants from crosses between efficient and inefficient varieties indicated complete dominance of the *Fe* allele. Absence of maternal inheritance was evidenced by similar performance of reciprocal crosses between the *Fe* and *fe* genotypes in the F_1 and F_2 generations.

Composition in aerial plant tissues, as conditioned by the inefficient gene, consisted of relatively higher pH, lower soluble iron, higher total iron, and lower potassium content. Although the data furnished no definite information as to identity of the primary causal agent, the assumption was made that relatively low solubility of iron in inefficient genotypes was induced by the comparatively high pH. On the other hand, relatively low potassium content might have accounted for the low hydrogen-ion concentration.

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